

Nos. 2023-2424, 2024-1176

**United States Court of Appeals
For the Federal Circuit**

SEAGEN INC.,

Plaintiff-Appellee,

v.

**DAIICHI SANKYO COMPANY, LTD., ASTRAZENECA
PHARMACEUTICALS LP, ASTRAZENECA UK LTD.,**

Defendants-Appellants.

On Appeal from the United States District Court for the Eastern
District of Texas, No. 2:20-cv-000337-JRG, Hon. Rodney Gilstrap

**NONCONFIDENTIAL OPENING BRIEF OF
DEFENDANTS-APPELLANTS**

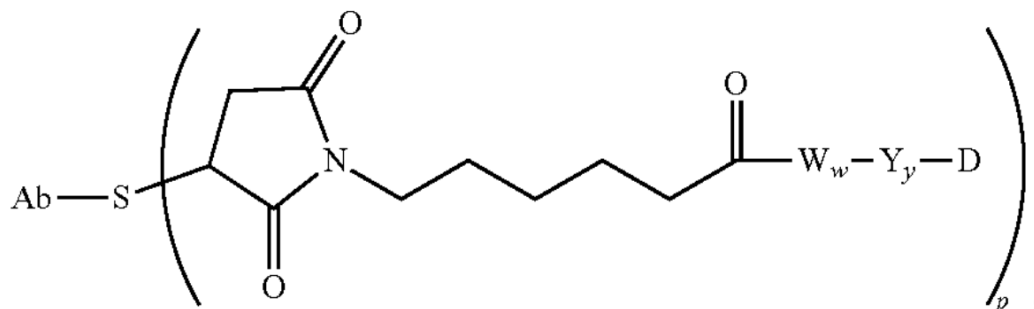
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March 22, 2024

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U.S. Patent No. 10,808,039, Claim 1 (Appx272)

1. An antibody-drug conjugate having the formula:

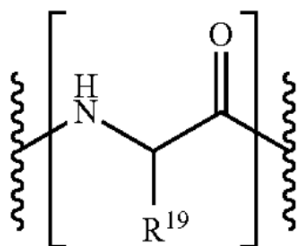


or a pharmaceutically acceptable salt thereof, wherein:

Ab is an antibody,

S is sulfur,

each $-W_w-$ unit is a tetrapeptide; wherein each $-W-$ unit is independently an Amino Acid unit having the formula denoted below in the square bracket:



wherein R^{19} is hydrogen or benzyl,

Y is a Spacer unit,

y is 0, 1 or 2,

D is a drug moiety, and

p ranges from 1 to about 20,

wherein the S is a sulfur atom on a cysteine residue of the antibody,
and

wherein the drug moiety is intracellularly cleaved in a patient from the antibody of the antibody-drug conjugate or an intracellular metabolite of the antibody-drug conjugate.

FORM 9. Certificate of Interest

Form 9 (p. 1)
March 2023

**UNITED STATES COURT OF APPEALS
FOR THE FEDERAL CIRCUIT**

CORRECTED CERTIFICATE OF INTEREST

Case Number 2023-2424, 2024-1176

Short Case Caption Seagen Inc. v. Daiichi Sankyo Company, Ltd.

Filing Party/Entity Defendants-Appellants Daiichi Sankyo Company, Ltd., AstraZeneca Pharmaceuticals LP, AstraZeneca UK Ltd.

Instructions:

1. Complete each section of the form and select none or N/A if appropriate.
2. Please enter only one item per box; attach additional pages as needed, and check the box to indicate such pages are attached.
3. In answering Sections 2 and 3, be specific as to which represented entities the answers apply; lack of specificity may result in non-compliance.
4. Please do not duplicate entries within Section 5.
5. Counsel must file an amended Certificate of Interest within seven days after any information on this form changes. Fed. Cir. R. 47.4(c).

I certify the following information and any attached sheets are accurate and complete to the best of my knowledge.

Date: 03/22/2024

Signature: /s/ Christopher N. Sipes

Name: Christopher N. Sipes

FORM 9. Certificate of Interest

Form 9 (p. 2)
March 2023

1. Represented Entities. Fed. Cir. R. 47.4(a)(1).	2. Real Party in Interest. Fed. Cir. R. 47.4(a)(2).	3. Parent Corporations and Stockholders. Fed. Cir. R. 47.4(a)(3).
Provide the full names of all entities represented by undersigned counsel in this case.	Provide the full names of all real parties in interest for the entities. Do not list the real parties if they are the same as the entities. <input checked="" type="checkbox"/> None/Not Applicable	Provide the full names of all parent corporations for the entities and all publicly held companies that own 10% or more stock in the entities. <input type="checkbox"/> None/Not Applicable
AstraZeneca Pharmaceuticals LP		AstraZeneca PLC
AstraZeneca UK Ltd.		AstraZeneca PLC
Daiichi Sankyo Company, Ltd.		See attached page.



Additional pages attached

FORM 9. Certificate of Interest

Form 9 (p. 3)
March 2023

4. Legal Representatives. List all law firms, partners, and associates that (a) appeared for the entities in the originating court or agency or (b) are expected to appear in this court for the entities. Do not include those who have already entered an appearance in this court. Fed. Cir. R. 47.4(a)(4).

☐ None/Not Applicable ☒ Additional pages attached

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5. Related Cases. Other than the originating case(s) for this case, are there related or prior cases that meet the criteria under Fed. Cir. R. 47.5(a)?

☒ Yes (file separate notice; see below) ☐ No ☐ N/A (amicus/movant)

If yes, concurrently file a separate Notice of Related Case Information that complies with Fed. Cir. R. 47.5(b). **Please do not duplicate information.** This separate Notice must only be filed with the first Certificate of Interest or, subsequently, if information changes during the pendency of the appeal. Fed. Cir. R. 47.5(b).

6. Organizational Victims and Bankruptcy Cases. Provide any information required under Fed. R. App. P. 26.1(b) (organizational victims in criminal cases) and 26.1(c) (bankruptcy case debtors and trustees). Fed. Cir. R. 47.4(a)(6).

☒ None/Not Applicable ☐ Additional pages attached

Certificate of Interest**(p. 4)****Response to Question 3 (Continued):**

Daiichi Sankyo Company, Ltd. has no parent corporations, and 10% or more of its stock is held by the Master Trust Bank of Japan Ltd. (trust account).

Response to Question 5 (Continued)

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**Statement Regarding Confidential Material Pursuant to Fed.
Cir. R. 25.1(e)(1)(B):**

Material has been redacted from pages 78, 80, and 81 of the Nonconfidential Opening Brief and pages Appx5-19, Appx20-31, and Appx32-49 of the addendum attached to the Nonconfidential Opening Brief. This material has been designated pursuant to the Protective Order entered in *Seagen Inc. v. Daiichi Sankyo Co., Ltd., et al.*, No. 2:20-cv-00337-JRG (E.D. Tex.). The redacted material relates to confidential product development and financial information that was placed under seal by the district court.

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STATEMENT OF RELATED CASES

No appeal from the proceedings below was previously before this or any other appellate court.

On January 16, 2024, the Patent Trial and Appeal Board found that Claims 1-5, 9, and 10 of U.S. Patent No. 10,808,039 (the “’039 Patent”)—the same claims at issue in this appeal—are unpatentable, *inter alia*, as anticipated (for lack of priority) and for lack of enablement. *Daiichi Sankyo, Inc., et al. v. Seagen Inc.*, PGR2021-00030, Paper 57 (P.T.A.B. Jan. 16, 2024); Appx2508-2593. On February 14, 2024, Seagen Inc. filed a request for Director review of that decision, which remains pending.

The ’039 Patent is also at issue in *Daiichi Sankyo, Inc. et al. v. Seagen Inc.*, No. 20-cv-1524-GBW (D. Del.), which has been stayed in favor of the district court proceeding from which this appeal arises. The Delaware action was administratively closed on March 25, 2022, in light of the stay.

Appellants are unaware of any additional pending cases that could directly affect or be directly affected by this Court’s decision in the pending appeal.

JURISDICTIONAL STATEMENT

The district court had jurisdiction under 28 U.S.C. §§ 1331 and 1338. The district court entered judgment on July 19, 2022. Appx50-52. The district court denied Appellants' motions for, *inter alia*, judgment as a matter of law ("JMOL") and a new trial on August 21, 2023. Appx32-49; Appx2448-2462. The district court also awarded Seagen supplemental damages and ongoing royalties. Appx2463-2477. On September 18, 2023, Appellants moved to amend the earlier judgment based on the new damages award. Appx2478. On September 20, 2023, Appellants filed a protective notice of appeal. Appx2491-2497. The district court granted Appellants' motion to amend in-part and entered an Amended Final Judgment on October 17, 2023. Appx1-4; Appx5-19; Appx20-31. Appellants timely appealed on November 16, 2023. Appx2498-2503; *see* 28 U.S.C. § 2107(a); Fed. R. App. P. 4. This Court has jurisdiction under 28 U.S.C. § 1295(a)(1).

INTRODUCTION

This Court has cautioned that “the purpose of the written description requirement is to prevent an applicant from later asserting that he invented that which he did not, and the requirement is particularly important when, as here, claims are added later during prosecution in response to development by others.” *Quake v. Lo*, 928 F.3d 1365, 1373 (Fed. Cir. 2019) (citation and internal quotation omitted). This warning is particularly applicable here, where Appellee Seagen Inc. seeks to rely on a 20-year-old patent application to support claims drafted 15 years later in order to capture what it admittedly did not invent: Appellants’ groundbreaking cancer therapy Enhertu.

Enhertu is an Antibody-Drug-Conjugate (“ADC”) that was first created by Daiichi Sankyo in 2011, and, after years of development by Daiichi Sankyo and AstraZeneca, approved by FDA as “Breakthrough Therapy” in 2019.

That same year, Seagen—who had learned of Enhertu from Daiichi Sankyo publications and unsuccessfully sought an Enhertu partnership—filed a patent application with claims specifically drafted

to cover Enhertu. To sidestep the prior art literature describing Enhertu, Seagen claimed priority to an ancestor application from 2004.

In stretching to cover Enhertu with new claims, Seagen departed dramatically from the 2004 priority disclosure. First, Seagen claimed a new and distinctive subgenus of ADC linkers—so-called Gly/Phe-only tetrapeptide linkers—that it had never seen or used before Enhertu and, accordingly, is nowhere described or exemplified in Seagen’s 2004 disclosure. To the contrary, Seagen’s 2004 application sets forth a maze of possible ADC constructions, encompassing a near-limitless set of possibilities, and yet never describes the Gly/Phe-only tetrapeptide linkers Seagen ultimately sought to claim once it learned of Enhertu. Indeed, each of the named inventors testified that they had never even seen an ADC with a Gly/Phe-only tetrapeptide linker before seeing Enhertu, and Seagen’s own expert at trial, Dr. Carolyn Bertozzi, described such linkers as a “leap” from the application’s disclosure. Because Seagen’s new claims are unsupported by the 2004 Application, they are anticipated by Daiichi Sankyo’s 2015-2016 publications on Enhertu.

Second, Seagen's claims were expanded to encompass ADCs incorporating and delivering any drug moiety, a dramatic expansion from the ADCs in the 2004 disclosure, all of which incorporate a narrow class of drugs (not used in Enhertu). No examples or teachings enable Seagen's broad claim scope. Moreover, Seagen's functionally-defined claims require ADCs that will successfully undergo a complex biochemical reaction—intracellular cleavage of the ADC drug unit in a human patient—that is not taught or enabled by the 2004 disclosure. All witnesses acknowledged the complexity and unpredictability of creating ADCs, and that it takes even the most sophisticated researchers years of work to come close with no guarantee of success. Even today, making and using ADCs like those claimed takes years of trial-and-error work by teams of expert researchers. Doing so in 2004 with no guidance or examples would have been simply unrealistic.

Seagen's patent issued in 2020. Minutes after issuance, Seagen sued Daiichi Sankyo in the Eastern District of Texas alleging willful infringement. After a trial, the jury found willful infringement, no invalidity, and awarded \$42 million in past damages.

The Patent Trial and Appeal Board recently determined that all Asserted Claims in Seagen's patent are unpatentable as anticipated (for lack of priority) and for lack of enablement. Seagen has indicated that it intends to appeal that decision to this Court.

Seagen's jury victory cannot square with the damning evidence of invalidity presented at trial. The district court's denial of JMOL for lack of anticipation (for lack of priority) and enablement is wrong and requires reversal. A finding in Appellants' favor on either argument would resolve this appeal. To the extent this Court reaches the issue, the district court's denial of JMOL or a new trial on damages issues was also improper and requires reversal or vacatur and remand.

STATEMENT OF THE ISSUES

1. Whether Appellants are entitled to judgment as a matter of law that claims 1-5, 9, and 10 of the '039 Patent are invalid as anticipated for lack of priority.

2. Whether Appellants are entitled to judgment as a matter of law that claims 1-5, 9, and 10 of the '039 Patent are invalid for lack of enablement.

3. Whether the jury's damages award should be set aside because there is insufficient evidence in the record to support it.

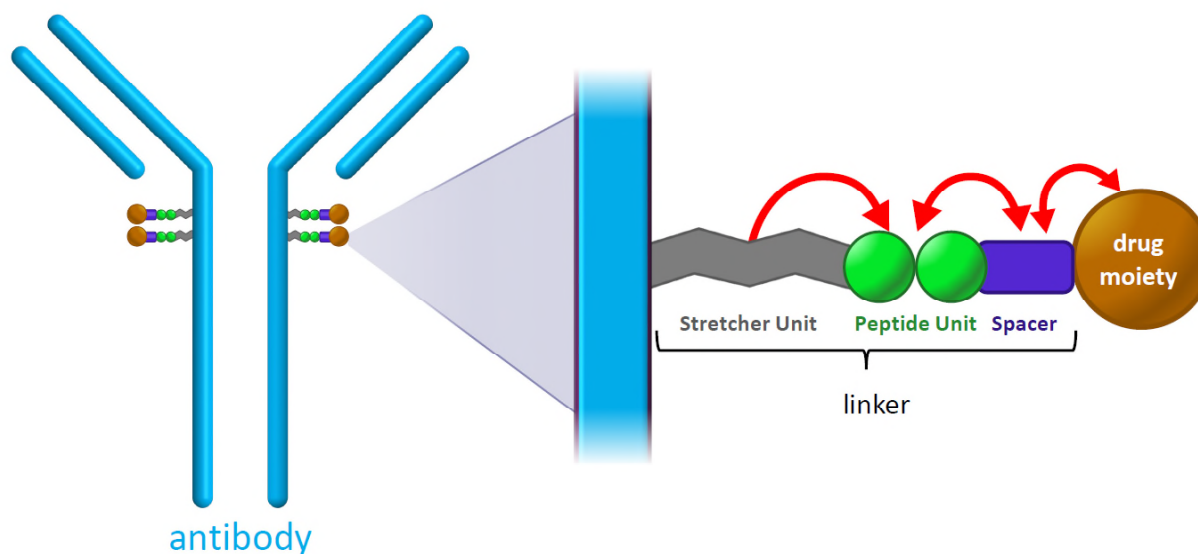
STATEMENT OF THE CASE¹

A. Antibody-Drug Conjugates (“ADCs”)

This appeal involves antibody-drug conjugates. ADCs are complex molecules that combine the cell-targeting abilities of antibodies with the cell-killing abilities of cytotoxic drugs, to deliver those drugs selectively to cancer cells, thereby treating cancer while sparing healthy cells in a way not possible with traditional chemotherapy. Appx3139-3140(11:16-13:3); Appx3225(58:17-59:17).

As illustrated in the simplified figure below, an ADC generally is composed of an antibody, which is connected to a drug moiety by a “linker.” Appx3140(13:4-14:13); Appx3225(58:17-59:2) (discussing Appx3548).

¹ Appellants present the facts in the light most favorable to Seagen, relying principally upon Seagen’s witnesses, and its patent and related filings.



Appx3548. A single antibody may be connected to multiple drug-linker units, and the number of drug-linkers per antibody is referred to as the drug-to-antibody ratio (or “DAR”). Appx3142(23:4-20) (discussing Appx3706); Appx 3197-3198(243:21-245:9) (discussing Appx3523).

In principle, when an ADC is administered to a patient, the antibody recognizes and targets specific cancer cells, bringing along with it the linker and drug moiety. Once the ADC reaches the target cell (and, for some ADCs, is internalized into the cell), the drug moiety is released to kill the target cell. Appx3141(18:2-19:22); Appx3225-3226(60:6-61:23). The manner of drug release can vary, depending on the design of the ADC and the type of linker used. For example, ADCs with “non-cleavable” linkers are internalized by the target cell and digested to release the

drug-linker, whereas ADCs with “cleavable” linkers are ingested and the linker is severed (“cleaved”) through a chemical reaction. Appx3225(60:6-22); Appx3140-3141(15:11-17:12); Appx3141(18:2-19:22).

ADCs are highly complex, and even small changes in the ADC or its components can significantly alter ADC properties and *in vivo* performance. Appx3225(58:23-59:17). As Seagen’s expert Dr. Carolyn Bertozzi acknowledged at trial, for ADCs designed to cleave, the design of the linker is “extremely important” because it must prevent cleavage and potential drug moiety release when the ADC is administered to the patient and circulating through the body, yet it must efficiently release the drug once the ADC reaches the target cell. Appx3140(13:20-14:13); Appx3106(209:18-210:5). An ADC may fail because the linker either releases the drug moiety too early or fails to release the moiety once inside the cell. Appx3140(13:20-14:13). Improper release can mean, among other things, that the target cells will not receive the intended dose of drug and may result in toxic exposure for the patient. Appx3106(210:6-17).

Relevant here, linkers may include chains of amino acids of various lengths called “peptide units.” Appx3107(214:7-215:7). A peptide unit

that includes two amino acids is called a *dipeptide* unit, one with three amino acids is called a *tripeptide* unit, and so on. Appx3144(31:20-32:8); Appx3243(131:14-132:1) (discussing Appx3621-3623). For some ADCs, the peptide linker may be designed to be cleaved by an enzyme in the target cell to release the drug moiety from the ADC. Appx3141-3142(20:21-21:12). Linkers also can include other chemical structures with names like “stretchers” and “spacers,” any of which affect overall ADC stability and function. Appx3141-3142(19:23-21:12).

Designing, synthesizing, and testing ADCs involves an unpredictable balance of competing considerations and factors. Appx3169(129:18-131:13) (discussing Appx8685-8691); Appx3140(13:4-14:13); Appx3143(25:10-25); Appx3123(278:1-10); Appx3220(37:18-24); Appx3296(78:15-79:15); Appx3185(196:7-25); Appx3225(58:21-59:17); Appx3240(119:12-120:10). For example, the near-infinite number of molecular components that could be drawn from to assemble an ADC frequently cannot be attached to one another. Appx3169(129:18-131:13); Appx3140(13:4-14:13); Appx3123(278:1-10); Appx3296(78:15-79:15); Appx3293(65:9-18); Appx3239(115:12-25). This is because all components of the ADC interact with each other in unpredictable and significant

ways, affecting the ultimate function of the ADC in the body. Appx3169(129:18-131:13); Appx8685; Appx3225(58:17-59:17).

All ADC components have unique properties that differ and must be accounted for. Appx3169(129:18-131:13); Appx3225(58:17-59:17). Even where the attachment of a drug is possible in an ADC, the ADC must still survive many biochemical hurdles to avoid unintended release and to ensure the drug moiety reaches the target cells. Appx3169(129:18-131:13); Appx3225(58:17-59:17). The linker must deliver the chosen drug moiety in the right way, at the right time, and in the right location with respect to the targeted cancer cells. Appx3140(15:11-24); Appx3169(129:18-131:13) (discussing Appx8685-8691); Appx3225(58:23-59:17). As of 2004—the priority date Seagen seeks to claim for the '039 Patent—the field of ADC design was nascent. Only one ADC had received FDA approval at the time—Pfizer's Mylotarg™ for lymphoma—which later had its approval withdrawn due to toxicity, with reapproval taking many years. Appx3107(213:19-214:6); Appx3140(15:11-24).

B. The 2004 Application

On November 5, 2004, Seagen filed U.S. Application No. 10/983,340 (“the 2004 Application”). *E.g.*, Appx5628-5639. The '039 Patent at issue

in this appeal claims priority to the 2004 Application via a long chain of divisional and continuation applications. Appx61. To avoid anticipation by Daiichi Sankyo's 2015-2016 publications describing Enhertu, Seagen contended at trial that the Asserted Claims of the '039 Patent are entitled to a priority date of no later than November 5, 2004—the filing date of the 2004 Application. Appx1327.

The 2004 Application describes ADCs that have a drug moiety selected from the narrow class of dolastatin/auristatin derivatives (“D/A-type drugs”), a narrow category of anti-cancer agents. Appx5642 (“[T]here is a clear need in the art for dolastatin/auristatin derivatives having significantly lower toxicity, yet useful therapeutic efficiency. These ... are addressed by the present invention.”); Appx3292(64:9-12); Appx3167(122:7-25); Appx3167(123:9-19); Appx3115(245:13-25); Appx3238(111:9-112:5); Appx3227(67:17-68:14); Appx3228(70:13-71:25).

Consistent with the “clear need” identified, the 2004 Application is titled “Monomethylvaline Compounds [i.e., D/A-type drugs] Capable of Conjugation to Ligands.” Appx5639; Appx3292(64:9-12); Appx3115(245:13-25); Appx3226(62:12-25); Appx3227(67:17-68:14). The abstract, as well as every embodiment, example, figure, and assay

disclosed in the 2004 Application involves D/A-type drug moieties. Appx5946; Appx3292(64:9-12); Appx3227(67:17-68:14); Appx3237(107:16-108:10) (discussing Appx3588); Appx3238(111:9-112:5) (discussing Appx3593).

The sections of the specification that describe “The Compounds of the Invention” and “The Drug Unit (Moiety)” address only D/A-type drugs. Appx5698-5719; Appx5731-5735. The “Background of the Invention” section makes reference to hundreds of different “drugs to kill or inhibit tumor cells in the treatment of cancer.” Appx5639-5641; Appx5680-5684; Appx3237-3238(108:11-112:5); Appx3588; Appx3591-3593; Appx3151(57:5-12) (discussing Appx3725). But the disclosure contains no examples or embodiments of any ADCs that employ any of these agents, instead they are described as options for co-therapy with the described D/A-type ADCs. Appx3237-3238(108:11-111:8) (discussing Appx3591-3592).

With respect to “The Linker Unit,” the 2004 Application discloses numerous categories and combinations of potential subcomponents, all of which are disclosed as being optional. Appx5719-5737. For example, “when present,” the “Stretcher unit (-A-)” is “capable of linking a Ligand

unit to an amino acid unit (-W-)." Appx5719; *see also* Appx5719-5722 (listing numerous potential stretcher units). Likewise, "when present," the "Spacer unit (-Y-)" can "link" any of the amino acid unit, the stretcher unit, or the ligand unit to the drug moiety, depending on which of the many optional components are present in the linker. Appx5726; *see also* Appx5726-5731 (listing numerous potential spacer units). The 2004 Application also mentions the "Ligand unit (L-)," which "includes within its scope any unit of a Ligand (L) that binds or reactively associates or complexes with a receptor, antigen or other receptive moiety associated with a given target-cell population." Appx5735; *see also* Appx5735-5737.

The 2004 Application also includes a short discussion of the "Amino Acid unit (-W-)." Appx5722-5725. "[W]hen present," the peptide unit "W_w" can be any of "a dipeptide, tripeptide, tetrapeptide, pentapeptide, hexapeptide, heptapeptide, octapeptide, nonapeptide, decapeptide, undecapeptide or dodecapeptide unit." Appx5722-5723; Appx3148(47:1-8); Appx3292(63:5-24). Thus, if a peptide unit is present at all in an ADC, it can include anywhere from one to twelve amino acid units. Appx3243(130:16-132:24). The 2004 Application identifies a list of 39 options for each amino acid in the peptide. Appx5723; Appx3293(68:15-

22). Once different isomers (spatial arrangements) of the identified amino acids are accounted for, this number grows to 83. Appx3243(130:16-132:24). Seagen did not dispute that this means that, just with respect to the tetrapeptide category, over 47 million different tetrapeptide units (83^4) are encompassed by the 2004 Application's disclosure. Appx3120(265:3-10); Appx3243(130:16-132:24).²

Despite its length, the application includes—at most—just three examples of tetrapeptides. Appx5725 (Table IX); Appx3148(48:8-21); Appx3243-3244(132:25-135:8) (discussing Appx3625-3627). The example Seagen focused on at trial provides one specific four-amino-acid sequence among the 47 million possibilities: glycine, phenylalanine, leucine (abbreviated “Leu” or “L”), glycine—which is a “Gly-Phe-Leu-Gly” or “GFLG” tetrapeptide.³ The other disclosed tetrapeptide sequences are

² Of course, if the other peptide lengths called out by the description are considered, the number of peptide linkers encompassed by the 2004 Application grows exponentially: for example, there are almost four billion pentapeptides (83^5).

³ Appx5725 (Table IX: “H,” “benzyl,” “isobutyl,” and “H”). The amino acid with isobutyl is leucine. Appx3148(48:8-21).

alanine (“Ala” or “A”), leucine, alanine, leucine—i.e., “ALAL”⁴—and “glycine serine valine glutamine”—i.e., “GSVQ.”⁵

There are no examples or description in the application of a tetrapeptide containing *only* glycine and phenylalanine, let alone a description of the subgenus of 81 Gly/Phe-only tetrapeptide linkers. Appx3148-3149(48:8-49:1); Appx3119(263:11-264:24); Appx3243-3244(130:16-133:3); *see also* Appx3306(119:5-16). Indeed, the tetrapeptide examples that Seagen did describe, including the Gly-Phe-Leu-Gly sequence on which it relies, were already known in the prior art. *See* Appx3308(127:17-128:11); Appx3280(14:17-15:19) (discussing Appx9859); Appx3143(26:6-27:20) (discussing Appx9859 and Appx3709 and acknowledging GFLG and ALAL “ha[d] significant potential liabilities”); *see also* Appx5554-5556.

The 2004 Application issued as U.S. Patent No. 7,498,298 on March 3, 2009, with claims directed to ADCs having certain D/A-type drug compounds. Appx3800; Appx4009-4018; Appx3116(252:4-21);

⁴ Appx5725 (Table IX: “methyl,” “isobutyl,” “methyl,” and “isobutyl”); Appx3294(71:7-20).

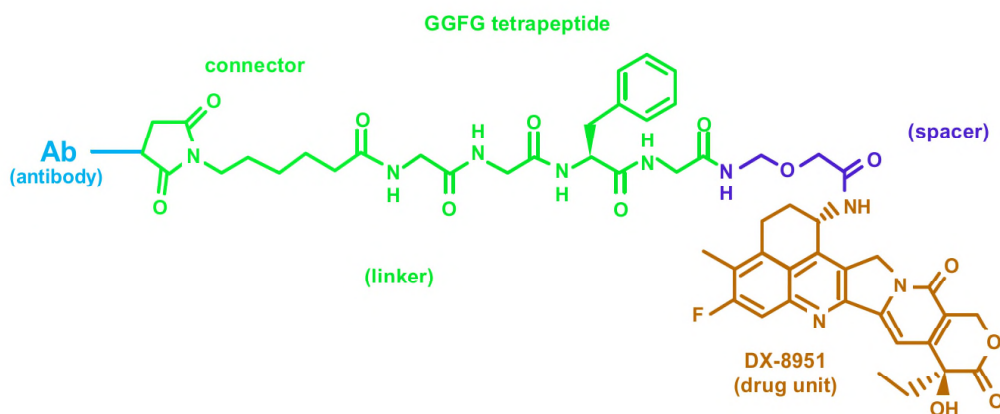
⁵ Appx5725; Appx3280(15:10-16:4).

Appx3228(71:2-25); Appx3167(122:3-25). Over the next decade, Seagen prosecuted continuation and divisional applications, all with claims involving D/A-type drug moieties. *See* Appx4020; Appx4225-4231 (Patent No. 7,994,135, filed in 2007); Appx4232; Appx4439-4443 (Patent No. 8,703,714, filed in 2011); Appx7316; Appx7592-7595 (Application No. 14/194,106, filed in 2014); Appx8161; Appx8424-8432 (Application No. 15/188,843, filed in 2016); Appx4444; Appx4652-4655 (Patent No. 10,414,826, filed in 2017). None of these applications or patents claim ADCs with drug moieties *other than* D/A-type drugs, and none claim ADCs with a tetrapeptide containing *only* glycine and phenylalanine. Appx3118-3120(260:7-265:18); Appx3292(64:9-12).

C. Enhertu

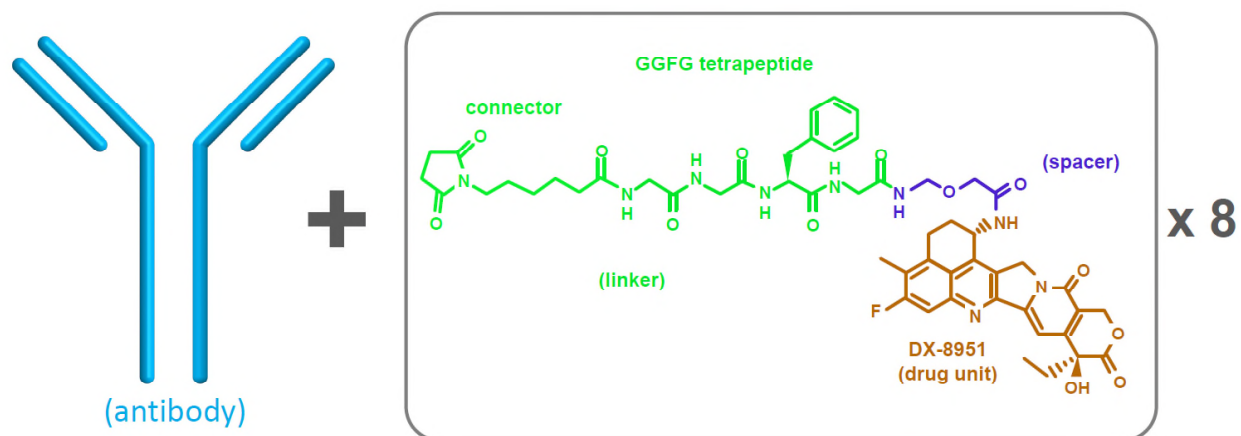
In 2011, Daiichi Sankyo scientist Dr. Hiroyuki Naito invented DS-8201—the ADC ultimately approved as Enhertu. Appx3196(239:9-16); Appx3196(240:3-10); Appx3198-3199(245:13-250:21) (discussing Appx3524-3528). In Enhertu, an antibody that targets cells expressing HER2 (a protein over-expressed in certain cancers) delivers a camptothecin-derivative (DXd) payload, which damages DNA and causes cell death. Appx8677; Appx9221; Appx8850.

The linker used in Enhertu contains a distinctive tetrapeptide unit having a glycine-glycine-phenylalanine-glycine amino acid sequence—also referred to as a “Gly-Gly-Phe-Gly” or “GGFG” tetrapeptide (shown in green below). Appx3196-3197(240:18-241:11) (discussing Appx3520); Appx3119(261:22-25); Appx3154(70:22-71:11) (discussing Appx3745); Appx3218(31:19-32:1). Attached to Enhertu’s Gly-Gly-Phe-Gly tetrapeptide is a spacer (purple), which is in turn attached to a camptothecin drug moiety (brown). Appx3196-3197(240:18-241:11) (discussing Appx3520); Appx3155(73:4-74:5) (discussing Appx3751).



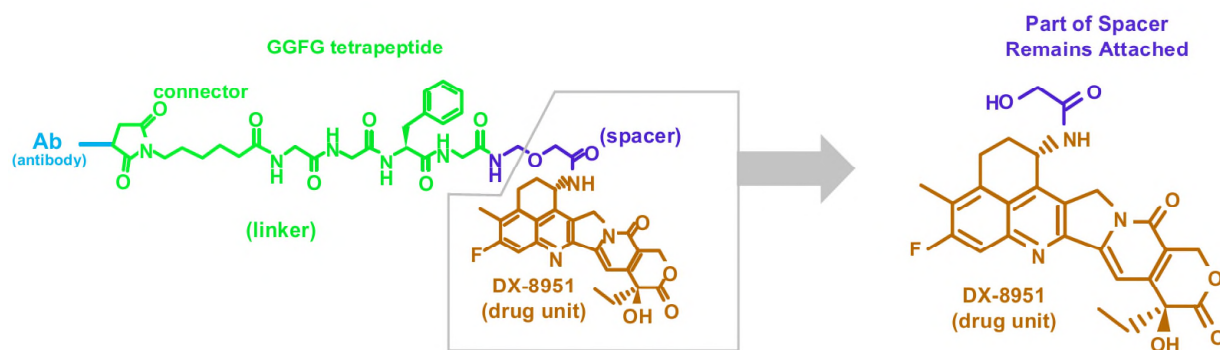
Appx3520.

Enhertu typically has eight linker-drug moiety constructs attached to each antibody, giving it a DAR of 8. Appx3197-3198(243:21-245:9) (discussing Appx3523); Appx3154-3155(72:7-73:3) (discussing Appx3749).



Appx3523.

After Enhertu is administered and reaches the cancer cell, it is cleaved between the tetrapeptide and spacer, after which a part of the spacer breaks down, releasing the drug unit with a part of the spacer still attached into the cell. Appx3197(242:1-25) (discussing Appx3521); Appx3155-3156(74:6-80:3) (discussing Appx3753-3760).



Appx3521.

FDA quickly recognized the impact and promise of Enhertu. FDA granted Fast Track Designation in 2016 and designated Enhertu a

“Breakthrough Therapy” one year later. Appx9220; Appx3185(193:14-194:6) (discussing Appx3504). After conducting Priority Review, the Agency approved Enhertu for treatment of unresectable or metastatic HER2-positive breast cancer at the end of 2019. Since then, Enhertu has also been approved for locally advanced or metastatic HER2-positive lung, gastric, and gastroesophageal cancers. Appx8665; Appx8850. These cancers are all aggressive and essentially “incurable.” Appx3287-3288(42:17-48:11) (discussing Appx3536-3538). Enhertu has proven uniquely effective, with many patients showing no evidence of disease following treatment. Appx3290(53:16-55:3).

Enhertu and its structure and mechanism of action were presented and published as early as December 2015. Appx3242(126:6-22); Appx3115(246:1-13); Appx8711-8723; Appx3121(271:3-13); Appx8724-8734; Appx8735. Seagen’s employees, including the named inventors, admitted at trial that they learned of Enhertu in December of 2015. Appx3115(246:1-247:24); Appx3120(265:25-266:22); Appx3218(32:17-23); Appx3221-3222(44:3-45:10). They further admitted that the first time they saw an ADC having a tetrapeptide made of only glycine and phenylalanine was in Enhertu. Appx3119-3120(261:15-266:2);

Appx3292(62:13-64:12); *see also* Appx3218-3219(31:19-33:3); Appx3221-3222(44:10-45:10); Appx3222-3223(48:3-50:6).

In the following years, Seagen came to refer to Enhertu as “Daiichi Sankyo’s proprietary payload and linker-payload technology” (Appx9012), and “Daiichi-Sankyo’s drug-linker[]” (Appx8903; Appx9047), which Seagen remarked was “[a] standout drug-linker” (Appx8951; Appx3246(144:2-16) (discussing Appx3640-3641)). Indeed, Seagen twice sought to partner with Daiichi Sankyo on its ADC drug-linker technology based on Enhertu. *See* Appx3187-3188(204:25-205:24); Appx8848-8849.

D. The 2019 Application

Three-and-a-half years after learning of Daiichi Sankyo’s “standout drug-linker” in Enhertu, and shortly after Daiichi Sankyo announced a multi-billion-dollar collaboration with AstraZeneca to further develop the drug, Seagen filed U.S. Application No. 16/507,839 (“the 2019 Application”), that ultimately led to the ’039 Patent. Appx61; Appx4657; Appx5053-5056. Conspicuously, the 2019 Application was the first time that Seagen sought claims to ADCs with drug moieties *other than* D/A-type drugs (that is, to claims that would cover Enhertu’s camptothecin drug moiety) or *with* tetrapeptides where each of the four amino acids

may be only glycine or phenylalanine (that is, “Gly/Phe-only tetrapeptides” including Enhertu’s Gly-Gly-Phe-Gly tetrapeptide). Notwithstanding this new claiming strategy, Seagen filed the 2019 Application as a continuation application, with a priority claim to the 2004 Application from fifteen years earlier. Appx61; Appx5053-5056.

During prosecution, to overcome a rejection, Seagen amended independent claim 1 to incorporate dependent claims 2-5, including the Gly/Phe-only and tetrapeptide limitations. Appx5102-5138; Appx5425-5433; Appx5102-5117; Appx5119-5138. The Examiner then rejected the amended claims as obvious over Dubowchik’s disclosure of ADCs having Gly-Phe-Leu-Gly tetrapeptides in view of Nogusa’s disclosure of “conjugates wherein the peptide spacer is either Gly-Gly-Phe-Gly or Gly-Phe-Gly-Gly.” Appx5554-5557. In response, Seagen argued that Gly-Phe-Leu-Gly tetrapeptides—the same sequence disclosed in the 2004 Application, on which it now relies as support for Gly/Phe-only tetrapeptides—“ha[d] significant potential liabilities,” and that a POSA would be “advised against” pursuing Gly/Phe-only tetrapeptides. Appx5588. Seagen did not point to any disclosure in its specification that addressed those significant potential liabilities or would modify the

POSA's view regarding those sequences. The Examiner then issued a notice of allowance. Appx5598-5605.

Seagen also sought patent protection in Europe through a counterpart patent application with a similar claim for priority. The European Examiner noted that, in contrast to Seagen's disclosure, the claims were no longer directed to D/A-type drugs and included a new Gly/Phe-only tetrapeptide. Appx5493-5499. Both limitations were found to contain added matter. Appx5493-5499. As the Examiner explained, "[t]he original application was a literal 'kitchen sink' of features" and "[t]o arrive at the subject-matter of the claims, it is necessary to carry out purposive selections from a number of lists." Appx5497. The Examiner stated that "[i]t is quite manifest that Applicants are trying to lay claim on Daiichi [Sankyo's Enhertu], a very promising chemotherapeutic drug, by mixing and matching features not disclosed in combination in the original application." Appx5497.

E. The '039 Patent

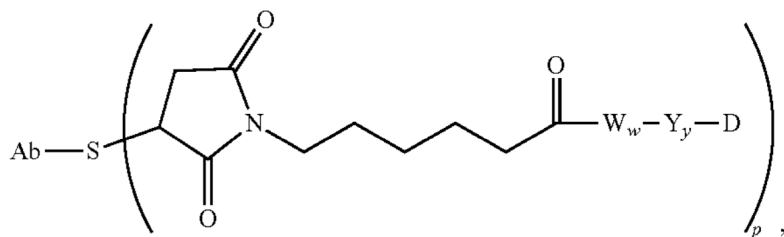
Unlike Seagen's earlier applications that languished in prosecution for years, Seagen requested expedited prosecution of the 2019

Application. Appx4781-4783. The '039 Patent issued on October 20, 2020, just over a year after filing. Appx61; Appx5602.

It is undisputed that the '039 Patent has the same “shared specification” as the 2004 Application. Appx2241. The claims of the '039 Patent, however, were newly added in 2019. *Supra* §D.

Claims 1-5 and 9-10 (“the Asserted Claims”) are directed to a broad, functionally defined genus of ADCs. Asserted claim 1 is representative:

1. An antibody-drug conjugate having the formula:



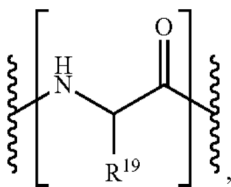
or a pharmaceutically acceptable salt thereof, wherein:

Ab is an antibody,

S is sulfur,

each $-W_w-$ unit is a tetrapeptide; wherein

each $-W-$ unit is independently an Amino Acid unit having the formula denoted below in the square bracket:



wherein R^{19} is hydrogen or benzyl,

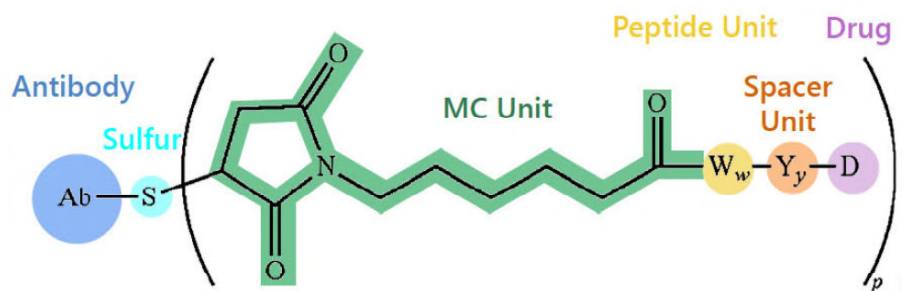
Y is a Spacer unit,

y is 0, 1 or 2,

D is a drug moiety, and

p ranges from 1 to about 20,
 wherein the S is a sulfur atom on a cysteine
 residue of the antibody, and
 wherein the drug moiety is intracellularly
 cleaved in a patient from the antibody of the
 antibody-drug conjugate or an intracellular
 metabolite of the antibody-drug conjugate.

Appx272(331:35-332:40). Seagen depicted the “claimed structure in claim 1” at trial as follows:



Appx3737; Appx3153-3154(67:2-69:3).

The claimed ADCs include a maleimidocaproyl or “MC” unit (green above), which was a well-established means of attaching components to an antibody as of the priority date. Appx3147(43:23-44:13) (discussing Appx3719); Appx3141(20:11-20); Appx3165(115:10-20). Bonded to the MC unit on both sides are four variable components—Ab, W_w , Y_y , and D—which must all be capable of attaching together to form an ADC. *See* Appx3106(209:18-210:17) (“[W]hat you want is the linker to be able to successfully attach a drug to an antibody so that the drug ... doesn’t come

off the antibody where you don't want it to."). Further, the claimed components must work together to yield an ADC in which the "drug moiety is intracellularly cleaved in a patient from the antibody" of the ADC, as claimed. *See* Appx3106(209:18-210:17) ("[Y]ou want the drug to be attached in a manner that it's stable when the antibody is in the circulation finding a tumor cell, but you want the linker to be able to let the drug go when it gets inside of a cell. That's very important.").

The claimed W_w peptide unit must be a Gly/Phe-only tetrapeptide. Each " W_w " unit "is a tetrapeptide" with four " W " units. Appx3147-3148(44:22-45:11) (discussing Appx3721). Each of the four W units must be the depicted formula, where " R^{19} is hydrogen or benzyl." When R^{19} is hydrogen, the W unit is glycine (abbreviated "Gly" or "G"), and when R^{19} is benzyl, the W unit is phenylalanine (abbreviated "Phe" or "F"). Appx3148(45:12-18) (discussing Appx3722); *see also* Appx3144(29:13-30:9). Thus, the W_w unit of claim 1 must be a four-amino-acid-long unit in which "each" amino acid is either glycine or phenylalanine—i.e., a Gly/Phe-only tetrapeptide. Appx3148(45:12-21) (discussing Appx3722); Appx3306(117:6-9); Appx3119(261:10-21).

F. District Court Proceedings

1. Pre-Trial Proceedings

During claim construction, Appellants argued that the claim term “D is a drug moiety” should be limited to D/A-type drugs. Appx1286-1287. Seagen urged an expansive “plain meaning” construction that “doesn’t restrict the invention to a particular drug moiety.” Appx3005(22:19-23); Appx1286-1287. The district court adopted Seagen’s broad construction. Appx1289; Appx1292. The district court reasoned, “[h]ad the patentee wanted to limit the claims to a particular drug or have particular limitations, it could have easily done so.” Appx1289.

2. Jury Trial

A five-day jury trial was held in April 2022. At trial, Appellants’ expert witness on anticipation and enablement was Dr. John Lambert, who helped pioneer the ADC field beginning in the 1980s and helped lead the team that created Kadcyla®, the first ADC approved to treat breast cancer. Appx3223-3225(51:8-57:23) (discussing Appx3544-3546). Dr. Lambert testified unequivocally that the Asserted Claims were neither described in nor enabled by Seagen’s 2004 Application or the ’039 Patent. Appx3238-3239(112:9-113:19); Appx3242(125:7-17); Appx3242(128:1-14); Appx3245(139:19-140:11). Dr. Lambert further

testified that, because the Asserted Claims are entitled to a priority date no earlier than 2019, they are anticipated by publications describing Enhertu. Appx3247(146:12-147:11) (discussing Appx3645-3646; Appx8711-8723).

The jury also heard from the named inventors of the '039 Patent, as well as other ADC scientists from Seagen. All named inventors testified they had never seen an ADC with a Gly/Phe-only tetrapeptide before Enhertu, let alone invented one. Seagen's named inventor, Dr. Peter Senter, admitted that "the first time [he] ever saw a G/F-only tetrapeptide in an ADC, it was in Daiichi Sankyo's Enhertu," that "Seagen wasn't the first to think of an ADC having a G/F-only tetrapeptide," and that, prior to 2019 (when the continuation application was filed), "no one within Seagen made G/F-only tetrapeptides." Appx3119(261:22-25); Appx3119(262:20-263:10); Appx3115(247:5-8); *see also* Appx3285(36:11-23); Appx3310(133:19-135:21); Appx3246-3247(144:17-145:8). The other named inventors, Dr. Toki, Dr. Kline, and Dr. Doronina testified to similar effect. Appx3292(62:13-63:4); Appx3292(63:25-64:12); Appx3293(67:17-19); Appx3295(74:9-12); Appx3297(81:13-84:9). Seagen's scientific directors, Dr. Jeffrey, Dr.

Alley, and Dr. Lyon, also agreed. Appx3218-3219(31:19-33:3); Appx3220(37:10-17); Appx3221-3222(44:3-45:10); Appx3222(45:15-46:5); Appx3222-3223(48:10-50:6).

Seagen's expert at trial was Dr. Carolyn Bertozzi. Dr. Bertozzi admitted that the 2004 Application does not disclose an ADC having a Gly/Phe-only tetrapeptide. Appx3306(119:5-16); Appx3307(123:16-19). Dr. Bertozzi testified that, in her view, the 2004 Application supported the Asserted Claims because it would be a "straightforward leap" to start with the disclosed Gly-Phe-Leu-Gly tetrapeptide—the Dubowchik prior art's "rejected" tetrapeptide—and then "combine" it with disclosures elsewhere in the specification and in the prior art to arrive at certain ADCs having a Gly/Phe-only tetrapeptide. Appx3149(50:21-51:7); Appx3298-3300(87:24-93:5). To support her opinion, Dr. Bertozzi pointed the jury to the named inventors' lab notebooks and other "highly confidential" documents, which include additional information not disclosed to the public, but which nevertheless fail to describe a Gly/Phe-only tetrapeptide. Appx3300-3301(93:6-97:8) (discussing Appx9859-9873; Appx9925-9926; Appx9829-9833). On enablement, in contrast to the unanimous view of nearly all witnesses that developing ADCs is

complex and unpredictable, Dr. Bertozzi stated that chemistries to make ADCs “are many decades old” and are taught “in a freshman laboratory.” Appx3302-3303(103:25-105:15). Dr. Bertozzi also opined on infringement with respect to Enhertu. Appx3152-3157(63:12-81:19) (discussing, *inter alia*, Appx9874; Appx9875-9887; Appx9888-9895, addressed *infra* §I).

The jury returned a general verdict of willful infringement, no invalidity on any ground, and past damages of nearly \$42 million. Appx53-60; Appx3407-3409(144:2-149:20). The district court subsequently awarded pre- and post-judgment interest, and an ongoing royalty through the expiration date of the '039 Patent. Appx2463-2477; Appx1-4.

3. Post-Trial Proceedings

At the close of the evidence, Appellants moved for JMOL on their invalidity and damages arguments. Fed. R. Civ. P. 50(a); Appx1815-1817; Appx3355-3356(315:10-318:25); Appx3357-3359(323:9-332:6). The district court denied JMOL in a single-sentence oral order and left all issues to the jury. Appx1856; Appx3360(335:21-336:6). Appellants timely renewed their JMOL motions after trial. Fed. R. Civ. P. 50(b); Appx2130-2131; Appx2168-2169. The district court again denied JMOL and held

that “sufficient evidence” existed to support the jury’s determinations without addressing Appellants’ arguments. *E.g.*, Appx7, Appx43, Appx46, Appx49.

G. Post-Grant Review Proceedings

In parallel with the district court, the Patent Trial and Appeal Board has also considered the ’039 Patent. Just *two months* after the ’039 Patent’s issue date (and well in advance of the statutory deadline) Daiichi Sankyo Inc. and AstraZeneca Pharmaceuticals LP petitioned for post-grant review (“PGR”) of the Asserted Claims. 35 U.S.C. § 321(c); *Daiichi Sankyo, Inc. v. Seagen Inc.*, No. PGR2021-00030 (P.T.A.B. Dec. 23, 2020), D.I. 1; Appx1915-1920. The petition raised the same invalidity issues that are presented in this appeal. Appx1917-1918. Indeed, the arguments, issues, witnesses, and evidence between the two proceedings overlap significantly. Appx2521-2536; Appx2547-2584; Appx2587-2591. Notwithstanding this near-immediate filing, resolution of the PGR was repeatedly delayed. Appx2509-2511 (summarizing procedural history).

On January 16, 2024—over three years after the petition was filed—the Board issued a final written decision concluding that the Asserted Claims are unpatentable as anticipated and for lack of

enablement. Appx2508-2593. Twenty-nine days after the Board's determination, Seagen sought Director review of the eligibility of the '039 Patent for PGR. *See* PGR2021-00030, D.I. 62.

SUMMARY OF THE ARGUMENT

I. *Anticipation For Lack of Priority.* The Court should reverse the district court's denial of JMOL of anticipation. Absent priority in the 2004 Application, the Asserted Claims are indisputably anticipated by the publication of Enhertu's structure in 2015 and 2016. And priority is lacking because no reasonable jury could find the 2004 Application to provide adequate written description support. Arriving at the claimed Gly/Phe-only tetrapeptide linkers requires arriving at an undescribed subgenus of 81 linkers by following a maze-like path through a long series of independent and optionally described selections, from a disclosure of millions or billions of possible linkers, without any blaze marks preferring one choice over another. The lack of written description is confirmed by unanimous testimony from the named inventors confirming they only learned of the claimed Gly/Phe-only linkers from seeing Enhertu, years after the priority date, and also from the testimony of Seagen's expert, who described the claimed linkers as a "leap" from the 2004 Application—an obviousness analysis this Court's precedents make clear is inadequate to establish written description support.

II. No Enablement. The district court’s denial of JMOL of no enablement should also be reversed. The Asserted Claims encompass a vast, functional genus of ADCs comprised of *any* drug moiety wherein the *any* drug moiety is “intracellularly cleaved in a patient.” The Supreme Court has instructed that “the more a party claims ... the more it must enable.” *Amgen Inc. v. Sanofi*, 598 U.S. 594, 613 (2023). In the complex and unpredictable field of ADC design, Seagen’s disclosure—which contains *zero* working examples and only limited guidance for a POSA—falls far short. Clear and convincing evidence at trial showed that, at every step of the way, a POSA seeking to make and use the full scope of the claimed ADCs would be required to engage in extensive and undue experimentation. Because no reasonable jury could find otherwise, and the district court’s contrary conclusion is unsupported, reversal is warranted.

III. Unsupported Damages. Should this Court find in Appellants’ favor on invalidity, this Court need not address damages. If the Court addresses the issue, however, the Court should reverse the district court’s denial of JMOL on damages, vacate the damages award, and remand. The only evidence supporting Seagen’s eight percent royalty

rate were license agreements that differ markedly in technological, commercial, and geographic scope from the hypothetical negotiation between Seagen and Appellants for a narrow, non-exclusive, U.S.-only license. The jury's award cannot stand on these grounds. *See, e.g., Lucent Techs., Inc. v. Gateway, Inc.*, 580 F.3d 1301, 1325 (Fed. Cir. 2009).

STANDARD OF REVIEW

This Court reviews the denial of JMOL according to the law of the regional circuit. *LifeNet Health v. LifeCell Corp.*, 837 F.3d 1316, 1322 (Fed. Cir. 2016). The Fifth Circuit reviews a denial of JMOL *de novo*. *In re 3 Star Properties, L.L.C.*, 6 F.4th 595, 607 (5th Cir. 2021). JMOL is appropriate if a “reasonable jury would not have a legally sufficient evidentiary basis to find for the party on that issue.” Fed. R. Civ. P. 50(a).

“[Compliance] with the written description requirement of § 112 ¶ 1 is a question of fact,” reviewed “for substantial evidence.” *Juno Therapeutics, Inc. v. Kite Pharma, Inc.*, 10 F.4th 1330, 1335-36 (Fed. Cir. 2021). “A patent [] can be held invalid for failure to meet the written description requirement based solely on the face of the patent specification.” *Centocor Ortho Biotech, Inc. v. Abbott Lab’s*, 636 F.3d 1341, 1347 (Fed. Cir. 2011).

“Whether a claim satisfies § 112’s enablement requirement is a question of law [that is] review[ed] *de novo*; however, in the context of a jury trial, [the Federal Circuit] review[s] the factual underpinnings of enablement for substantial evidence.” *Trustees of Boston University v. Everlight Electronics Co.*, 896 F.3d 1357, 1361 (Fed. Cir. 2018).

Similarly, this Court “review[s] the jury’s determination of the amount of damages, an issue of fact, for substantial evidence.” *Lucent Techs.*, 580 F.3d at 1310. “Substantial evidence requires more than a mere scintilla,” and this Court “must review the record as a whole, taking into consideration evidence that both justifies and detracts from the jury’s decision.” *Cordis Corp. v. Bos. Sci. Corp.*, 658 F.3d 1347, 1357 (Fed. Cir. 2011).

ARGUMENT

I. The Asserted Claims Are Anticipated by Enhertu Because They Lack Written Description Support in the 2004 Application.

At trial, Seagen argued that Enhertu meets every limitation of the Asserted Claims of the '039 Patent. *See, e.g.*, Appx3152-3157(62:25-81:19); Appx3172-3173(144:11-145:11) (discussing Appx8735). It is also undisputed that Daiichi Sankyo's 2015-2016 publications—including the 2016 Clinical Cancer Research article (Appx8711-8723; Appx9875-9887; Appx9241-9253), the 2016 Cancer Science article (Appx8724-8734; Appx9888-9895), and Daiichi Sankyo's 2015 Poster (Appx8735; Appx9874), each of which describe Enhertu's chemical structure and are prior art to Seagen's 2019 Application—disclose every limitation of the Asserted Claims. *See* Appx3154(70:22-71:11) (discussing Appx3745, Appx9877); Appx3156(80:6-24) (discussing Appx3762; Appx9874; Appx9883; Appx9890); Appx3152(63:12-24) (citing Appx9875-9887, Appx9888-9895, Appx9874); Appx3247(146:7-147:11) (discussing Appx3646-3647, Appx8711-8723). Accordingly, if the Asserted Claims cannot claim priority to the 2004 Application, they are invalid as anticipated by any one of the Enhertu publications. *See, e.g., Arthrex, Inc.*

v. Smith & Nephew, Inc., 35 F.4th 1328, 1344 (Fed. Cir. 2022) (affirming anticipation based on a lack of adequate written description in the priority document used to antedate prior art); *Bristol-Myers Squibb Co. v. Ben Venue Labs, Inc.*, 246 F.3d 1368, 1378 (Fed. Cir. 2001) (“It is axiomatic that that which would literally infringe if later anticipates if earlier.”).

For the Asserted Claims to claim priority to the 2004 Application, that Application must disclose the claimed invention “in the manner provided by section 112(a).” 35 U.S.C. § 120; *Tech. Licensing Corp. v. Videotek, Inc.*, 545 F.3d 1316, 1326 (Fed. Cir. 2008). Among other things, the 2004 Application must meet the written description requirement of Section 112(a).

This Court has emphasized that “the requirement is particularly important when, as here, claims are added later during prosecution in response to development by others.” *Quake*, 928 F.3d at 1373. “[T]he purpose of the written description requirement is to prevent an applicant from later asserting that he invented that which he did not,” *id.* (cleaned up), and it serves to “ensure that the scope of the right to exclude, as set forth in the claims, does not overreach the scope of the inventor’s

contribution to the field of art as described in the patent specification.” *Ariad Pharms., Inc. v. Eli Lilly & Co.*, 598 F.3d 1336, 1353-54 (Fed. Cir. 2010) (en banc). “[T]he hallmark of written description is disclosure.” *Id.* at 1351.

At trial, Appellants demonstrated by clear and convincing evidence that the 2004 Application does not satisfy the written description requirement with respect to the Asserted Claims. No reasonable jury could find otherwise. First, the specification says nothing about the now-claimed Gly/Phe-only tetrapeptides, which the named inventors acknowledged they were not even aware of until seeing Enhertu in 2015, and there are no blaze marks that would lead a POSA to understand that the named inventors were in possession of the narrow subset of Gly/Phe-only tetrapeptides from among the vast sea of possible linkers encompassed by the 2004 Application. Indeed, what little disclosure of tetrapeptide sequences exists in the Application includes amino acids beyond glycine and phenylalanine, contradicting the now-claimed limitation to Gly/Phe-only tetrapeptides. Second, Seagen’s reliance on the hindsight-driven testimony that the claimed tetrapeptides would be a “straightforward leap” from disclosure of the 2004 Application fails as a

legal matter to provide written description support and serves to confirm that the 2004 Application itself lacks the required disclosure; the expert's improper reliance on disclosure outside the Application to support her testimony similarly confirms the gaps in that Application. Accordingly, this Court should reverse the denial of Appellants' JMOL motion.

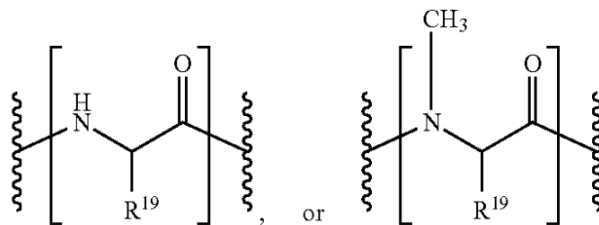
A. There is no description of ADCs having the claimed Gly/Phe-only tetrapeptides and no blaze marks pointing to such ADCs.

The absence of an adequate description of ADCs with the newly claimed Gly/Phe-only tetrapeptides is evident from the 2004 Application itself. Despite Seagen's more than 200-page specification, only a few short paragraphs are devoted to " W_w ," the peptide unit. Appx139-140(65:45-68:12); Appx5722-5725.⁶ That discussion indicates that inclusion of any peptide unit at all in an ADC is ***optional***. Appx139(65:46, 65:55); Appx5722-5725. Indeed, apart from a vague suggestion that "[u]seful [peptide] units ***can*** be designed and optimized" to "liberate" the drug, there is no specific advantage disclosed for using

⁶ Parallel citations to the '039 Patent are provided for convenience. It is undisputed that the 2004 Application has the same "shared specification" as the '039 Patent. Appx2241; *see supra* §E.

peptide units in ADCs. Appx140(67:57-62); Appx139(66:43-47); Appx5724-5725.

When a peptide unit is present, the specification discloses that it can vary significantly in length, ranging from one or two (“dipeptide”) to twelve (“dodecapeptide”) amino acid units long. Appx139(65:50-53); Appx5723. The word “tetrapeptide” is used only twice in the entire specification, and both times it appears alongside a laundry list of other peptide unit lengths. Appx139(65:50-53) (“...tripeptide, tetrapeptide, pentapeptide, hexapeptide...”); Appx5723; Appx140(67:62-63) (“In one embodiment, —W_w— is a dipeptide, tripeptide, tetrapeptide or pentapeptide.”); Appx5725. Indeed, the specification highlights the variability of the “W_w” unit and states that “each [amino acid] unit independently has the formula denoted below in the square brackets, and w is an integer ranging from 0 [i.e., no peptide unit at all] to 12”:



Appx139(65:53-64); Appx5723.⁷ The specification does not suggest that tetrapeptides are better (or worse) than any of the other peptide unit lengths described. Appx139(65:53-64); Appx5723; *see also* Appx3297(83:1-23).

The variability of amino acids in the peptide (the “—W—” units) exponentially increases the number of possible different “W_w” peptide linkers encompassed by the disclosure. Appx139(65:53-65); Appx5723. The specification describes 39 different amino acid alternatives for the “R¹⁹” group, which determines the identity of the —W— unit. Appx139(65:65-66:8) (1-30); Appx139(66:8-42) (31-39); Appx5722; Appx3148(45:25-47:20) (discussing Appx3723); Appx3298(88:14-22) (discussing Appx9395). Dr. Kline, a named inventor of the ’039 Patent, admitted the specification lists “every natural amino acid that I can imagine ... as well as a plethora of non-coded amino acids.” Appx3297(83:1-84:9); *see also* Appx3293-3294(68:9-70:4) (the specification discloses “a significant number of amino acids”). Once isomeric forms of the amino acids are taken into account, the number of

⁷ The disclosure thus would encompass peptide linkers containing just a single amino acid, in addition to those between two and twelve amino acids in length.

options for each amino acid in the peptide chain—which includes anywhere from 1 to 12 amino acids—grows to 83 different amino acids. Appx3243(130:16-132:24).

Taken together, the number of peptides encompassed by the disclosure of the specification is overwhelming. Accounting for tetrapeptides alone, any of 83 amino acid units could be selected for each of the four links in the tetrapeptide chain. Appx3120(265:3-6); Appx3243(132:18-24). In other words, as Seagen’s named inventor Dr. Senter admitted, the specification describes a genus of “***over 47 million***” tetrapeptides (83^4). Appx3120(265:3-6); Appx3243(132:18-24). Longer lengths, of course, have exponentially more possibilities.⁸

Of the handful of example peptide unit sequences of *any* length, the vast majority are not tetrapeptides. Appx139-140(66:47-67:55) (listing dipeptides and tripeptides); Appx5724-5725; Appx3244(133:4-135:8) (discussing Appx3625-3627); Appx5724-5725.⁹ The specification

⁸ For example, the specification encompasses almost 4 billion (83^5) different pentapeptides.

⁹ The specification discloses seventeen “illustrative” peptide units of varying lengths: eleven dipeptides, three tripeptides, and three tetrapeptides. See Appx139-140(66:47-68:12); Appx5724-5725; Appx3244(133:13-6) (discussing Appx3625).

contains, at most, only three exemplary tetrapeptide sequences. Appx140(67:35-50) (Table IX); Appx5725; Appx3148(48:3-21); Appx3243-3244(132:25-133:3); Appx3280(14:2-8). As Seagen's named inventors and Dr. Bertozzi admitted, **none** of the examples are Gly/Phe-only tetrapeptides. Appx3119(263:3-264:7); Appx32924(63:5-64:12); Appx3295(73:23-74:12); Appx3297(83:1-84:9); Appx3306(119:5-16).

There is nothing in the specification suggesting that tetrapeptides are preferred over other peptide lengths. Nor is there anything in the specification that suggests that Gly/Phe-only tetrapeptides are a possible subgenus of tetrapeptides at all, much less preferred over any other tetrapeptide (or other peptide length). As named inventor Dr. Kline conceded, "nothing in [the specification] points you one way or the other towards or away from the gly/phe-only containing tetrapeptides." Appx3297(83:19-23).

This Court has long held that written description disclosure of a broad genus fails to support patent claims to a narrow subgenus unless the disclosure contains "blaze marks" that would "provide[] adequate direction which reasonably would lead persons skilled in the art to the sub-genus of the [claim]." *Fujikawa v. Wattanasin*, 93 F.3d 1559, 1570

(Fed. Cir. 1996) (cleaned up); *see also Idenix Pharm. LLC v. Gilead Scis. Inc.*, 941 F.3d 1149, 1164 (Fed. Cir. 2019). Whether blaze marks are present and where they would lead a POSA must be assessed “from the standpoint of one with no foreknowledge of the specific [claim].” *In re Ruschig*, 379 F.2d 990, 995 (C.C.P.A. 1967). “In the absence of such blazemarks, simply describing a large genus of compounds is not sufficient to satisfy the written description requirement as to particular species or subgenuses.” *Fujikawa*, 93 F.3d at 1571.

Here, no reasonable jury could find the 2004 Application contained adequate blaze marks to the claimed ADCs having Gly/Phe-only tetrapeptides. As noted above, the 2004 Application does not even express a preference for tetrapeptide linkers, instead simply including “tetrapeptide” in an undifferentiated list of peptide lengths stretching from zero to twelve. Appx139-140(65:50-64, 67:62-63); Appx5723; Appx5725.

Moreover, the specification is equally devoid of any blaze marks pointing to Gly/Phe-only tetrapeptides. The specification instructs that each unit of a peptide may be occupied by any of 39 amino acid units, all of which are listed together in the specification. Appx139(65:65-66:42);

Appx5723. This translates to 83 possible selections for each unit when accounting for isomers. Appx3243(130:16-132:24). Nothing in the specification guides a POSA toward using only glycine or phenylalanine at each position in a tetrapeptide linker. As named inventor Dr. Kline conceded, the specification contains no blaze marks to Gly/Phe-only tetrapeptides: “It’s not called out.” Appx3297(83:13-18). Further, nothing in the specification would have led a POSA to exclude the other 80 amino acid choices at each and every position of the tetrapeptide.¹⁰

In short, the written description never mentions or suggests even one Gly/Phe-only tetrapeptide—let alone the claimed subgenus of 81 Gly/Phe-only tetrapeptides—and the few examples of tetrapeptides in the specification are not Gly/Phe-only. As such, they cannot provide a blaze mark to a claim that excludes those examples and includes only undisclosed subject matter. *See Regents of the Univ. of Minnesota v. Gilead Scis. Inc.*, 61 F.4th 1350,1358 (Fed. Cir. 2023) (lack of written description where the priority documents blaze a trail “that runs close by

¹⁰ *See also Novartis Pharms. Corp. v. Accord Healthcare, Inc.*, 38 F.4th 1013, 1016 (Fed. Cir. 2022) (“For negative claim limitations ... the specification [must] describe[] a reason to exclude the relevant element.”).

the later-claimed tree,” but “do[] not direct one to the proposed tree in particular, and do[] not teach the point at which one should leave the trail to find it.” (quoting *Fujikawa*, 93 F.3d at 1571)); *Novozymes v. DuPont Nutrition Biosciences APS*, 723 F.3d 1336, 1349 (Fed. Cir. 2013) (affirming JMOL of no written description: “one searches the 2000 application in vain for the disclosure of even a single species that falls within the claims or for any ‘blaze marks’ that would lead an ordinarily skilled investigator toward such a species among a slew of competing possibilities”).

The lack of written description is further confirmed by Seagen’s own inventor testimony. This Court has made clear that inventor testimony may illustrate the absence of written description. *See Nuvo Pharm. (Ireland) Designated Activity Co. v. Dr. Reddy’s Labs. Inc.*, 923 F.3d 1368, 1381 (Fed. Cir. 2019) (“Although inventor testimony cannot establish written description support where none exists in the four corners of the specification, it illuminates the absence of critical description in this case.”); *Idenix*, 941 F.3d at 1164 (citing admission of named inventor that claimed sub-genus was only conceived after priority document in support of holding no written description as a matter of law).

This makes sense: admissions by the named inventors that they had not conceived the claimed subject matter confirms that the subject matter was not described. *See Fiers v. Revel*, 984 F.2d 1164, 1171 (Fed. Cir. 1993) (“[O]ne cannot describe what one has not conceived.”).

Just such testimony exists here. The named inventors of the '039 Patent uniformly testified they had never made or even seen an ADC having a Gly/Phe-only tetrapeptide as of the claimed 2004 priority date. Appx3119-3120 (261:15-266:2); Appx3292(62:13-64:12); Appx3293(67:17-19); Appx3295(74:8-12); Appx3297(81:13-84:9); *see also* Appx3218-3219(31:19-33:3); Appx3220(37:10-17); Appx3221-3222(44:18-46:5); Appx3222(48:10-50:6).

Indeed, Seagen's lead named inventor, Dr. Senter, conceded that the first time he saw a Gly/Phe-only tetrapeptide, it was from learning about Enhertu. Appx3119(261:22-25). He further acknowledged that the specification “does not disclose anywhere the particular subgenus of 81 tetrapeptides with only G and F” and that the original 2004 Application “didn't specify Daiichi Sankyo's G/F-only amino acid sequence because Seagen wasn't aware of it at the time of the filing.” Appx3119(263:11-14); Appx3119(264:15-20).

In short, the specification's silence as to the now-claimed Gly/Phe-only tetrapeptides, and its description instead of only tetrapeptide examples falling outside the claim, is itself clear and convincing evidence that the written description requirement is not satisfied. *See Centocor*, 636 F.3d 1341, 1347 (Fed. Cir. 2011) ("A patent [] can be held invalid for failure to meet the written description requirement based solely on the face of the patent specification."); *Univ. Of Rochester v. G.D. Searle & Co.*, 358 F.3d 916, 927 (Fed. Cir. 2004) (same). No reasonable jury could find that the 2004 Application provides support for the Asserted Claims.

B. Seagen's "straightforward leap" testimony at trial does not provide substantial evidence of written description for the Asserted Claims.

At trial, Seagen did not dispute that the 2004 Application lacked disclosure of a single ADC within the scope of the '039 Patent claims and conceded that none of the Application's examples describe a Gly/Phe-only tetrapeptide. Instead, Seagen argued that the tetrapeptides of the claims are a "straightforward leap" from one of the three example tetrapeptides in the specification. The district court, in turn, pointed only to this theory as providing sufficient evidence for the jury's verdict. Appx43. However, Seagen's "straightforward leap" theory fails to provide substantial

evidence of written description as a matter of law, and indeed confirms that failure.

Seagen's theory, which it presented through its expert Dr. Bertozzi, is precisely the sort of "maze-like path" through multiple alternatives that this Court condemned in *Regents*. See 61 F.4th at 1357. Dr. Bertozzi started by noting that the specification uses the word "tetrapeptide" and, in a separate location, describes glycine and phenylalanine (among many options) as amino acids. Appx3298-3299(88:7-89:6). Dr. Bertozzi then jumped to the tetrapeptide examples in the specification (ignoring the larger group of exemplary dipeptides and tripeptides). Appx3299(89:7-14); see also Appx3148-3149(48:3-49:1); Appx3149(49:20-50:6); Appx3149(50:21-51:7). She then focused on just one exemplary tetrapeptide, the one having a Gly-Phe-Leu-Gly amino acid sequence, ignoring the other tetrapeptide examples. Appx3299(89:15-23); Appx3148-3149(48:3-49:1); Appx3149(49:20-50:6); Appx3149(50:21-51:7). Acknowledging her selected example was not a Gly/Phe-only tetrapeptide, Dr. Bertozzi nevertheless asserted that "three out of four amino acids in this peptide are G or F" and that it would have been "a straightforward leap to go from GFLG, as an example presented in the

patent, to a peptide that's all G and F." Appx3148-3149(48:22-49:1); Appx3149(50:25-51:2); Appx3299(90:9-92:18).

Dr. Bertozzi identified nothing in the specification that indicated a preference for a tetrapeptide over the other peptide lengths mentioned in the specification; to the contrary, her testimony was simply that "[t]he tetrapeptide is *an option* for this amino acid unit." Appx3148(47:7-8) (emphasis added). Nor did she testify that, in the list of 39 possible amino acids set forth in the specification (83 with isomers), there is an indicated preference for glycine or phenylalanine; again, glycine is "one of the options" and phenylalanine "another option." Appx3148(47:16-20).

Likewise, in selecting the specific example from which to make her "leap" to Gly/Phe-only tetrapeptides, Dr. Bertozzi identified nothing in the specification that identified that example as preferred over any other, let alone as a preferred starting point from which to make modifications in the disclosed peptide, so as to leap to a Gly/Phe-only tetrapeptide linker. Rather, in Dr. Bertozzi's words, the specification simply "shows an example of a tetrapeptide that one could use to make an ADC linker." Appx3148(48:5-7). She did not assert that the POSA would arrive at a Gly/Phe-only tetrapeptide starting with any other example.

Dr. Bertozzi's testimony is thus a hindsight-driven selection, from a vast array of options set forth in the specification, of just those choices that lead to one of the claimed Gly/Phe-only tetrapeptides. While Dr. Bertozzi focused on tetrapeptides, the specification allows for peptides of any length from one or two to twelve amino acids (or indeed, no peptide at all). While Dr. Bertozzi focused on the disclosure of glycine and phenylalanine as possible amino acids in a peptide linker, the specification identifies at least 37 (really 80) other amino acids for use. And while Dr. Bertozzi selects one example as the jumping off point for her proposed "straightforward leap," the specification contains more than a dozen other examples of peptide linkers, the vast majority of which are not tetrapeptides.

Dr. Bertozzi's ability, after the fact, to select from among the many possible options set forth in the specification just the right ones to get to the claimed invention does not provide substantial evidence that the specification shows possession by the applicants, at the time of filing in 2004, of that invention. In this Court's words, "[f]ollowing this maze-like path, each step providing multiple alternative paths, is not a written description of what might have been described if each of the optional

steps had been set forth as the only option.... [A]ll those optional choices do not define the intended result that is [the claim].” *Regents*, 61 F.4th at 1357; *Ruschig*, 379 F.2d at 995 (written description cannot be established by “[w]orking backward from” the claim).

Moreover, even were it proper to rely upon such hindsight-driven choices among competing options in the specification (it is not), Seagen’s theory would still fail because Seagen must still rely upon a “straightforward leap” to get from the specification to the claimed Gly/Phe-only tetrapeptides. That such a “leap” is necessary confirms that the specification itself does not show possession. This Court has repeatedly held that possession may not be shown by modifying the specification’s disclosure, even if such modifications may have been obvious to a POSA. *Novozymes*, 723 F.3d at 1350 (“The question before us is not whether one of ordinary skill in the art presented with the 2000 application would have been enabled to take those final steps, but whether the 2000 application ‘discloses the [variants] to him, specifically, as something appellants actually invented’” (quoting *Ruschig*, 379 F.2d at 995)); *see also Ariad*, 598 F.3d at 1352 (“[A] description that merely renders the invention obvious does not satisfy the [written description]

requirement.” (citation omitted)); *Lockwood v. Am. Airlines, Inc.*, 107 F.3d 1565, 1572 (Fed. Cir. 1997) (“It is not sufficient for purposes of ... written description ... that the disclosure, when combined with the knowledge in the art, would lead one to speculate as to modifications that the inventor might have envisioned, but failed to disclose.”).¹¹

Put another way, that the specification may contain an example that, in Dr. Bertozzi’s words, is just a “leap” from the claimed invention does not suffice to provide a blaze mark to the claimed invention itself. In *Fujikawa*, for example, this Court observed that the application’s “preferred embodiments do blaze a trail through the forest; one that runs close by Fujikawa’s proposed tree.” 93 F.3d at 1571. The application nonetheless failed to provide written description support because it “does not direct one to the proposed tree in particular, and does not teach the point at which one should leave the trail to find it.” *Id.* Similarly, in *Ruschig*, the disclosure of the application did “get[] close” to the

¹¹ Moreover, Dr. Bertozzi’s litigation theory that a POSA would have “leap[ed]” and “combin[ed]” a Gly-Phe-Leu-Gly tetrapeptide to reach a Gly/Phe-only tetrapeptide is contrary to Seagen’s argument to the Examiner during prosecution that Dubowchik’s failed Gly-Phe-Leu-Gly tetrapeptide “advised against” using a Gly/Phe-only tetrapeptide. Appx5588.

challenged claim, such that disclosing the claim would be “a simple change.” 379 F.2d at 995. Nonetheless, the application failed to provide written description support: “The trouble is that there is no such disclosure, easy though it is to imagine it.” *Id.*

Thus, under long-settled law, Dr. Bertozzi’s concession that arriving at the claimed invention requires a “leap” from the specification’s disclosure alone concedes that the specification itself does not show possession of the invention claimed. Even if the claimed invention were within leaping distance of the specification, the specification does not describe that leap, let alone describe Dr. Bertozzi’s selected example as a jumping off point.

Additional problems undermine Seagen’s theory. First, while Dr. Bertozzi attempted to explain how the specification might be expanded to *include* tetrapeptides made entirely with glycine and phenylalanine, she did not identify anything in the specification that describes *excluding* linkers made with any other amino acid at any position. The very example she uses for her “leap” includes leucine in addition to glycine and phenylalanine. Appx3149(50:21-51:2). Even if it would be “straightforward to find the 81 G and F tetrapeptides using the

disclosure of the '039 patent,” as Dr. Bertozzi testified (Appx3149(50:21-51:2)), that would not suffice to support a claim that includes **only** such linkers, while excluding all others, including those—such as the Gly-Phe-Leu-Gly linker from which Dr. Bertozzi makes her leap—that are actually described in the specification.

To be sure, a specification may provide support for limiting a claim to particular disclosed embodiments. But here, the claims are limited to embodiments never disclosed in the specification; every example in the specification is excluded. Because the specification fails even to mention Gly/Phe-only tetrapeptides, it cannot provide support for treating such peptides as a distinct class or for limiting the claimed ADCs to ones having such linkers, rather than any of the linkers actually described in the specification. As this Court has observed in the context of claim construction, “it is unlikely that an inventor would define the invention in a way that excluded the preferred embodiment, or that persons of skill in this field would read the specification in such a way.” *Hoechst Celanese Corp. v. BP Chemicals Ltd.*, 78 F.3d 1575, 1581 (Fed. Cir. 1996). Yet this is exactly how Seagen attempts to understand the 2004 Application.

Seagen’s “straightforward leap” theory does nothing to demonstrate that a POSA would understand the 2004 Application to show possession of a claimed sub-genus of ADCs (those with Gly/Phe-only tetrapeptide linkers) that both goes beyond the disclosure of the 2004 Application and also excludes every example identified in the Application. *See Idenix*, 941 F.3d at 1165 (“tens or hundreds of thousands of possible nucleosides” described, “yet the compound in question is conspicuously absent”). The exclusion from the Asserted Claims of all the examples described in the 2004 Application, shows that those claims contradict the Application’s teaching concerning the claimed invention, further demonstrating the lack of written description for the later-crafted Asserted Claims. *See In re Bimeda Rsch. & Dev. Ltd.*, 724 F.3d 1320, 1324 (Fed. Cir. 2013) (affirming rejection for lack of written description where claim’s exclusion of a particular compound was contrary to the specification’s disclosure); *Novartis*, 38 F.4th at 1016 (“For negative claim limitations ... the specification [must] describe[] a reason to exclude the relevant element.” (citation and internal quotation marks omitted)).

And second, to support her “step” to Gly/Phe-only tetrapeptides, Dr. Bertozzi impermissibly “combin[ed]” selected portions of the

specification with other documents outside the specification. Appx3298-3300(87:24-93:5). The written description requirement is “an objective inquiry into the four corners of the specification from the perspective of a person of ordinary skill in the art.” *Ariad*, 598 F.3d at 1351. And while “[t]he knowledge of ordinary artisans may be used to inform what is actually in the specification,” it cannot be used “to teach limitations that are not in the specification.” *Rivera v. Int’l Trade Comm.*, 857 F.3d 1315, 1322 (Fed. Cir. 2017). Dr. Bertozzi defied this settled law by relying upon passages from two research articles, “highly confidential” company documents, and a “highly confidential” email from 2020—all outside the four corners of the specification—to support her testimony that a POSA would understand that a tetrapeptide example of the specification could be modified to be Gly/Phe-only. Appx3299-3301(92:9-97:2); Appx9829-9833; Appx9925-9926; Appx9847-9858; Appx9229-9240; Appx9228. Dr. Bertozzi’s assertion that “there are clear blazemarks in the patent application combined with the prior art, [and] the research” that was not in the application (Appx3300-3301(96:23-97:2)), fails on its own terms to show written description support in the specification itself.

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Because a reasonable jury could not find the specification either to describe ADCs with the claimed Gly/Phe-only tetrapeptides or to set forth blaze marks leading a POSA to such ADCs, the Asserted Claims lack priority and are anticipated. Reversal is required.

II. The Asserted Claims Are Invalid for Lack of Enablement.

The enablement requirement embodied in 35 U.S.C. § 112(a) ensures that, “upon the expiration of the patent, the knowledge of the invention inures to the people, who are thus enabled without restriction to practice it.” *Amgen*, 598 U.S. at 605 (quoting *United States v. Dubilier Condenser Corp.*, 289 U.S. 178, 187 (1933) (cleaned up)). “[T]he more a party claims for itself the more it must enable.” *Id.* at 616. An inventor may attempt to claim an entire class of an alleged invention, but if so “the patent’s specification must enable a person skilled in the art to make and use the entire class” without unreasonable experimentation. *Id.* at 610; *Baxalta Inc. v. Genentech, Inc.*, 81 F.4th 1362, 1367 (Fed. Cir. 2023) (citing *Amgen*, 598 U.S. at 610-12).

Appellants showed by clear and convincing evidence at trial that the Asserted Claims are invalid for failure to satisfy the enablement requirement. There is no substantial evidence to the contrary. First, it

was not possible as of 2004 to design and synthesize each of the vast number of species of ADCs falling within the scope of the Asserted Claims. But even assuming it was, to then somehow test all such ADCs to determine whether the drug moiety is “intracellularly cleaved in a patient” as claimed using the iterative process proposed by Seagen and Dr. Bertozzi would by definition constitute impermissible trial-and-error experimentation, including under *Amgen* and its progeny. Second, and relatedly, evaluation of evidence presented at trial in view of the *Wands* factors shows that there is no substantial evidence to support the jury’s conclusion that the Asserted Claims satisfy the enablement requirement. Accordingly, the Court should reverse the denial of JMOL and remand with instructions to enter judgment of invalidity.

A. The ’039 Patent provides, at most, an impermissible roadmap for trial-and-error experimentation.

The Asserted Claims of the ’039 Patent purport to claim a monopoly over a nearly limitless number of functionally claimed ADCs. The vast breadth of the claimed genus is evident from the plain language of claim 1. Appx272(331:35-332:40); *see supra* §E. The claimed ADCs can include combinations of *any* drug moiety and *any* spacer (if one is present at all) attached to an antibody. Anywhere from “1 to about 20” linkers can be

attached per antibody. The only restraints on the scope of the claim are the MC unit (which was well-known in the art), the Gly/Phe-only tetrapeptide (which itself is an 81-member subgenus that the named inventors testified they never made or even contemplated until they saw Enhertu), and, importantly, the functional requirement that “the drug moiety is intracellularly cleaved in a patient,” which itself requires that the ADC remain stable until it reaches the cell and is internalized such that only then will the drug moiety be cleaved.

The specification, however, falls well short of enabling a POSA to make and use the innumerable ADCs encompassed by the claims. Critically, the specification provides **zero** working examples of the claimed invention, and it is silent as to the creation of a linker having a Gly/Phe-only tetrapeptide, let alone how such a tetrapeptide can be combined with *any* drug, or the other claimed ADC components and then achieve intracellular cleavage. Appx3240(119:3-120:4). To the contrary, the specification’s limited examples and guidance all involve D/A-type drugs and so-called “PAB” spacers, which represent only an infinitesimal fraction of the universe of *all* drugs claimed by Seagen. Appx177-183(141:60-154:14) (listing “exemplary” ADCs with D/A-type drugs). For

all non-D/A-type drugs, the specification provides no guidance or examples to teach the POSA how to attach the drug to a linker and conjugate it to an antibody. *E.g.*, Appx3220-3221(40:16-41:5); Appx3238-3240(112:20-118:19).

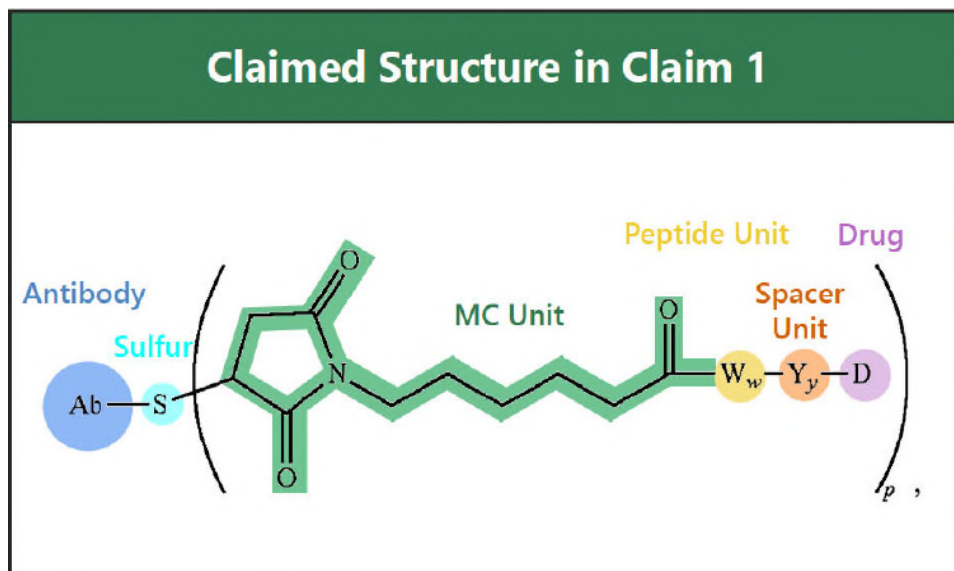
Evidence from Seagen's own witnesses at trial makes clear that impermissible trial-and-error experimentation would be required to practice the full scope of the claims. Seagen's expert, Dr. Bertozzi, conceded that "[t]here are a lot of moving parts in an ADC, and there's no generic formula for success." Appx3169(129:18-131:1). This is because "[h]ow the antibody chemically connects to the drug, the number of drugs on each antibody, the stability of the chemical linker, and whether the payload works in a particular tumor type are all important facets to the technology," and thus "[b]alancing all those needs when creating an ADC ... is a relatively large drug development undertaking." Appx3169(130:2-13); Appx3169(130:15) ("[I]t's definitely a significant undertaking, yes."). Similarly, as Appellants' expert Dr. Lambert explained, "the chemical elements of the linker and drug moiety can all interact with each other" and there is a "really complicated interplay of properties." Appx3225(58:20-59:17). Indeed, "it is a whole iterative process to try to

figure out whether you can put a chemical handle on a given drug,” (Appx3239(115:12-25)), and then further, “it is difficult to determine what the release drug moiety is in cancer cells, and it can even vary from cell to cell,” (Appx3241(123:18-124:10)).

Dr. Bertozzi nevertheless maintained that “any one of those many drugs that are listed” in the specification of the ’039 Patent—just a small subset of the claimed universe of *all* drug moieties—could be used in the claimed invention without undue experimentation based on a conclusory assertion that chemistries required to create ADCs “are many decades old” and taught “in a freshman laboratory.” Appx3302-3303(103:25-105:15). Even taking Dr. Bertozzi’s testimony at face value, she was only referring to the “many drugs that are listed” in the specification—not *all* drugs, as claimed.

Dr. Bertozzi also did not point to anything in the ’039 Patent that teaches the POSA to make and use *all* drugs, connected to *any* spacer (which is optional), connected specifically to a Gly/Phe-only tetrapeptide (which is not described in the specification, much less invented or contemplated by the named inventors, and would require a prerequisite

“leap” by the POSA to achieve), then connected to an MC unit, which itself must be conjugated to an antibody, as she admitted was claimed:



Appx3737; Appx3153(67:2-68:9). To the contrary, it was undisputed at trial that the specification does not disclose *even a single example* that meets the claims to guide the POSA in making the full scope of the vast genus claimed. Appx3306-3307(119:5-16, 123:16-19); Appx3119(263:3-264:7); Appx3292-4(63:5-64:12); Appx3295(73:23-74:12); Appx3297(83:1-84:9).

The problem for the POSA is compounded by the uncertain task of determining, assuming a particular ADC can be synthesized, whether that ADC would “intracellularly cleave[] in a patient” to release free drug inside the targeted cell as claimed, particularly in light of the

specification's lack of guidance as to which ADC components could do so. Appx272(331:35-332:40); Appx1292; Appx1318-1319. Seagen argued that in 2004 there existed "cell death" and "a wide selection of other known *in vivo* and *in vitro* assays that [a POSA] could have used to determine whether a particular ADC was intracellularly cleaved in a patient." Appx2263-2264 (citing Appx171-172; Appx174-176; Appx198-199; Appx71-72; Appx76-77; Appx80-86; Appx3139-3140(12:09-13:3); Appx3143-3244(28:2-31:19); Appx3141(18:2-19:4); Appx3106(209:18-210:17); Appx9302; Appx9304-9305; Appx9307; Appx9828; Appx8701-8703; Appx8843; Appx8845; Appx9242-9246; Appx9249; Appx9839-9812; Appx9844). Even if Seagen were correct on this point, it does not save the Asserted Claims because to practice the full scope of the claims, a POSA would have to first make the innumerable ADCs covered by the claims and then assay each of them to determine whether the drug moiety is intracellularly cleaved in a patient. Appx3123(278:1-10); Appx3169(129:18-131:13); Appx3220(37:18-24); Appx3220-3221(40:16-41:5); Appx3240-3241(119:12-122:15); Appx3296(78:15-79:15).

Amgen demonstrates the lack of enablement here. There, the patentee claimed a genus of antibodies that could "bind" amino acid

residues on a protein, and “block” the protein from binding other receptors. *Amgen*, 598 U.S. at 602. The number of antibodies that might perform these functions was virtually limitless because “[a]ntibodies are incredibly diverse” and “there may be as many unique antibodies as there are stars in the galaxy.” *Id.* at 599-600. The patentee only disclosed 26 example antibodies that performed the functions. *Id.* at 602-03. To determine what *other* antibodies would meet the claims, the patentee only provided the POSA with two methods—the “roadmap” and “conservative substitution” methods—for individually testing antibodies to see how they perform. *Id.* at 613-14. Because these testing methods did little more than “leave [the POSA] to ‘random trial-and-error discovery,’” the patentee’s broad claims were not enabled. *Id.* at 615-16.

The Asserted Claims are even more problematic than those at issue in *Amgen*. ADC development, like antibody development, is indisputably complex and unpredictable. *Id.* at 600. Whereas the patentee in *Amgen* sought to “claim for itself an entire universe of antibodies” that could “bind” and “block” as claimed, *id.* at 613, here, the claimed ADCs include an antibody as just one subcomponent of the claimed genus, to which a linker having *any* or no spacer and *any drug* can be conjugated with a

Gly/Phe-only tetrapeptide that can be “intracellularly cleaved in a patient” as claimed. The patentee in *Amgen* provided 26 examples that met the claims; Seagen has provided none. To the contrary, the named inventors of the ’039 Patent had not even conceived of such Gly/Phe-only ADCs and considered their contribution to be specific D/A-drugs and their derivatives in ADCs and the chemistry for attaching them to linkers. Appx3119(262:20-24); Appx3292(64:9-12).

It is not permissible to backfill the specification’s lack of disclosure with Dr. Bertozzi’s conclusory testimony. *See Idenix*, 941 F.3d at 1159 (“[I]t would be improper to rely on a POSA’s knowledge ... to fill the gaps in the specification. ‘It is the specification, not the knowledge of one skilled in the art, that must supply the novel aspects of an invention in order to constitute adequate enablement.’” (citing *Genentech, Inc. v. Novo Nordisk A/S*, 108 F.3d 1361, 1366 (Fed. Cir. 1997))). Rather, to identify ADCs that meet the Asserted Claims—even if Seagen’s “assays” were used and the underlying chemistries well known—the POSA would have to carry out a laborious, trial-and-error research assignment to test each candidate ADC to determine if it “intracellularly cleave[s] in a patient”—just like the “roadmap” and “conservative substitution” methods found to

be non-enabling in *Amgen*. As Dr. Bertozzi admitted, “it’s definitely a significant undertaking.” Appx3169(130:2-15). *Amgen*, 598 U.S. at 609.

Baxalta likewise illustrates the problem with the Asserted Claims. There, the patentee claimed “all antibodies” that bind and increase the activity of a given enzyme. *Baxalta*, 81 F.4th at 1363, 1366. In contrast to the “*millions* of potential candidate antibodies” that might meet the claim, “the written description disclose[d] the amino acid sequences for only *eleven* antibodies with the two claimed functions.” *Id.* at 1366 (emphasis original). The patentee argued that its patent disclosed a process that “predictably and reliably generates new claimed antibodies every time it is performed.” *Id.* at 1367. But this Court held that, even so, the POSA still would need to “make candidate antibodies and screen them to determine which ones perform the claimed functions,” which is “the definition of trial and error.” *Id.* By Dr. Bertozzi’s admission, Seagen’s “assays” would require the same type of iterative, trial-and-error experimentation for each ADC within the vast genus of claimed ADCs. Appx3169(129:18-131:13); Appx3123(278:1-10); Appx3220(37:18-24); Appx3220-3221(40:16-41:5); Appx3240-3241(119:12-122:15); Appx3296(78:15-79:15).

B. The *Wands* factors confirm that undue experimentation is required to practice the full scope of the claims.

This Court may also consider the *Wands* factors in determining whether undue experimentation is required to practice the full scope of the claims. *See In re Wands*, 858 F.2d 731 (Fed. Cir. 1988). Those factors include “(1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims.” *Id.* at 737. Applying these factors to the clear weight of evidence at trial confirms the ’039 Patent leaves a POSA to engage in painstaking and undue, trial-and-error experimentation to make and use the claimed invention.

1. Extensive experimentation would be required for a POSA to make and use the full scope of the claimed genus (Factors 1, 8).

The Asserted Claims encompass an “innumerable number of ADCs” having unique combinations of the claimed components. Appx3238-3239(112:9-113:19); Appx3155(73:4-74:5); *see also, e.g.*, Appx139-140(65:45-68:12) (“The Amino Acid Unit”); Appx140-141(68:13-70:65)

(“The Spacer Unit”); Appx142-145(71:18-77:22) (“The Drug Unit (Moiety)”); Appx107-108(2:43-3:7) (“cytotoxic or cytostatic agents”); Appx122-124(31:39-35:5) (“chemotherapeutic agents”); and “chemotherapeutic agent[s]”; Appx117-118(22:21-24:14), Appx130(48:24-50), Appx135-136(57:29-59:19), Appx149-161(86:10-110:8) (“Antibodies which comprise Ab”). Determining whether all of the ADCs within the scope of the claims meet the functional intracellularly-cleaving-in-a-patient limitation is impossible without trial-and-error experimentation, which requires stability in circulation, the ability to internalize, and then cleavage only once within the tumor. *See supra* §II.A. It is undisputed that creating an ADC is a “significant undertaking.” Appx3169(130:11-15); Appx3169(129:18-131:13).

2. Seagen’s disclosure provides no working examples or meaningful direction (Factors 2, 3).

Seagen’s specification lacks the examples and guidance necessary to enable a POSA to practice the claimed invention without undue experimentation. Appellants presented un rebutted evidence at trial that the ’039 Patent does not teach which combinations of antibodies, linkers, drugs, and other components will result in ADCs that will “intracellularly cleave[] in a patient” as claimed, nor does the ’039 Patent

teach how to determine whether a potential embodiment of the claimed genus of ADCs will “intracellularly cleave[] in a patient.” Appx3240-3241(119:3-120:4, 123:18-124:10). Nor does the ’039 Patent provide guidance on whether the broad range of ADCs covered by the claims will be stable in circulation, where they will cleave (assuming they cleave at all), or whether the “free drug” (or some other portion) of the ADCs cleaves. Appx3239(116:11-21); Appx3240(119:16-120:4).

Despite the incalculable number of ADCs encompassed by Seagen’s broad claim, the specification provides ***zero*** working examples of the claimed invention. Appx3240(119:3-120:4); *see also* Appx3148-3149(48:5-49:1). Rather, the overwhelming and un rebutted testimony at trial was that no one at Seagen, including the named inventors, had ever made or seen an ADC that embodied the Asserted Claims until 2015, when Enhertu was made public. Appx3309(130:20-131:4). Seagen’s failure to provide any working examples weighs against enablement, particularly in light of the wide breadth of Seagen’s claims. *See Idenix*, 941 F.3d at 1161.

The only guidance Seagen points to in its specification are isolated teachings that Seagen suggests relate to “various types of drug classes.”

Appx2260. But as Seagen appears to acknowledge, “this information appears in the context of attaching auristatin and dolastatin derivatives” (Appx2260)—which are only a small subset of the genus of *all* drugs encompassed by Seagen’s claims. Appx3155(73:4-74:6); Appx3237(107:16-108:2). This information provides no meaningful direction to the POSA seeking to make and use the ADCs of the Asserted Claims in light of the undisputed trial testimony from Seagen’s witnesses that “[t]here is no one linker that will work for every drug” (Appx3296(78:15-79:15)), and “[n]ot all drugs can be linked to antibodies, period” (Appx3220(37:18-24)). *See also* Appx3301(97:18-20); Appx3169(129:18-131:13); Appx3123(278:1-10); Appx3220-3221(39:13-41:5); Appx3285(34:3-36:10); Appx3238-3239(112:9-113:19).

Seagen appears to concede that “the specification does not include precise instructions on how to attach every type of drug to the claimed linker” because, in its view, this information is already within the knowledge of a POSA. *See* Appx2259. Even assuming a POSA had such knowledge at the time (they did not), Seagen’s attempt to rely on the POSA to remedy the specification’s glaring lack of disclosure is improper. *Genentech*, 108 F.3d at 1366 (“It is the specification, not the knowledge

of one skilled in the art, that must supply the novel aspects of an invention in order to constitute adequate enablement.”). Consequently, the prior art cannot remedy Seagen’s failure to enable the full scope of its broad, functional genus claim in its disclosure, particularly in the highly unpredictable field of ADC design and development. *See infra* §II.B.3; *Genentech*, 108 F.3d at 1366 (“[W]hat is well known in the art ... [is] not a substitute for a basic enabling disclosure.”).

3. ADC design is highly unpredictable, and the art does not resolve this unpredictability (Factors 4, 5, 6, and 7).

The creation of ADC technologies is typically done by highly specialized teams in a research and development setting. Appx3106(212:6-13); Appx3139(9:9-10:9); Appx3233(90:11-21). The level of ordinary skill of the individuals working in such teams is usually high, consistent with the complex nature of the molecules being developed. Appx3237(107:3-15); Appx3303(105:5-8).

Nevertheless, to defend enablement, Dr. Bertozzi took the dubious position that chemistries to make ADCs “are many decades old,” “well-known,” and taught “in a freshman laboratory.” Appx3302-3303(103:25-105:15). Nearly every witness at trial, however, agreed that ADCs are

complex and the process of inventing them is unpredictable and not routine. Appx3123(278:1-10); Appx3106(209:18-210:17); Appx3169(129:18-131:13); Appx3220(37:18-24); Appx3220-3221(39:13-41:5); Appx3225(58:21-59:17); Appx3238-3239(112:9-113:19); Appx3239-3240(116:6-117:5); Appx3284(30:17-25); Appx3285(34:3-36:10). As Dr. Kline testified, “it’s not a simple field. There are a lot of moving parts.” Appx3296(78:15-79:15).

The undisputed history of ADC discovery also contradicts Dr. Bertozzi’s suggestion that it is routine. The world’s largest pharmaceutical companies have spent decades working to develop ADCs, with only limited success. Appx3140-3141(15:11-18:1) (discussing Appx3691-3692); Appx3168-3169(127:20-129:9); Appx3248-3250(152:23-157:18). Indeed, as of Seagen’s alleged 2004 priority date, there was only *one* FDA-approved ADC. Appx3107(213:19-214:6). As Dr. Senter testified, “in the early 2000s” the “pharmaceutical companies that thought [ADCs were] going to be the cure for cancer had walked away.” Appx3106(211:15-212:13).

* * *

Taken together, the large breadth of the claims, absence of working examples, limited direction and guidance provided, the unpredictability in the field, and the extensive amount of experimentation necessary show that the Asserted Claims are not enabled. The district court's judgment to the contrary should be reversed.

III. Seagen Did Not Provide a Legally Sufficient Factual Basis to Support the Jury's Damages Award.

The jury's award of \$41.82 million in past damages was based on the eight percent royalty rate for Enhertu sales advocated by Seagen's damages expert, Ms. Distler. To meet Seagen's burden to prove damages, *see Lucent*, 580 F.3d at 1324, Ms. Distler, in turn, relied on several agreements that are purportedly comparable to a hypothetical October 2020 negotiation between Seagen and Appellants for the '039 Patent. Appx3322(181:15-182:11); Appx3333(226:14-15); *see also* Appx50; Appx28-29; Appx15. But Ms. Distler failed to account for the significant technological, commercial, and geographic differences between these agreements and the narrow hypothetical negotiation over the '039 Patent. Because those agreements are not comparable to the hypothetical negotiation here, they fail to provide a legally sufficient factual basis for the jury's award. The district court accordingly erred in denying JMOL,

and because the jury's damages award is against the weight of the evidence, a new trial on damages is needed. *Lucent*, 580 F.3d at 1310 (a damages award is not based on substantial evidence if it is "clearly not supported by the evidence, or based only on speculation or guesswork" (cleaned up)).

A. Seagen's damages case depends on licenses that are not commensurate with the hypothetical negotiation.

To support a damages award, a license must be "commensurate with what the defendant has appropriated." *ResQNet.com, Inc. v. Lansa, Inc.*, 594 F.3d 860, 872 (Fed. Cir. 2010); *Wordtech Sys., Inc. v. Integrated Networks Sols., Inc.*, 609 F.3d 1308, 1319 (Fed. Cir. 2010) (licenses are not "sufficiently comparable" where "they arose from divergent circumstances and covered divergent material"). "[T]here must be a basis in fact to associate the royalty rates used in prior licenses to the particular hypothetical negotiation at issue in the case." *Uniloc USA, Inc. v. Microsoft Corp.*, 632 F.3d 1292, 1317 (Fed. Cir. 2011); *see also Lucent*, 580 F.3d at 1325 (reviewing court examines whether proffered licenses "are sufficiently comparable to the hypothetical license"). Seagen's evidence falls well short.

At trial, Ms. Distler testified that the hypothetical negotiation between Seagen and Appellants would result in “a non-exclusive license to freely make, use, and sell Enhertu ... in the U.S.” Appx3313(147:14-15). To that end, Ms. Distler relied on two prior license agreements: (1) a [REDACTED] license information (Appx8857-8898), and (2) a [REDACTED] license information (Appx9051-9217), to establish a bargaining range for the hypothetical negotiation (*see* Appx3321(179:9-16); Appx3333(226:14-15)). But, in stark contrast to the hypothetical negotiation, these agreements are, among other things, “exclusive” (Appx8857; Appx9051), and “worldwide” (Appx8857). *See also* Appx3331(217:7-22)).

Moreover, both alleged comparator agreements license multiple patents and patent families. *See* Appx8860; Appx3330(214:6); Appx8894-8896; Appx9163-9167; Appx9171. Ms. Distler, however, “failed to address the extent to which these other patents contributed to the royalty rate” in the licenses. *Apple Inc. v. Wi-LAN Inc.*, 25 F.4th 960, 973 (Fed. Cir. 2022). Without isolating the value of the patents in a comparator agreement to match the technological scope of the hypothetical license, there is not a sufficient “basis in fact to associate the royalty rates used

in prior licenses” to a given hypothetical negotiation. *Uniloc*, 632 F.3d at 1317; *see also Apple*, 25 F.4th at 973-74 (finding damages expert failed to address the value of other patents licensed in comparator licenses, and the extent to which their absence would decrease the royalty rate in the hypothetical negotiation); *ResQNet.com*, 594 F.3d at 869 (“This court has long required district courts ... to exercise vigilance when considering past licenses to technologies *other* than the patent in suit.”); *Wordtech Sys.*, 609 F.3d at 1320 (“[C]omparisons of past patent licenses to the infringement must account for ‘the technological and economic differences between them.’” (quoting *ResQNet.com*, 594 F.3d at 873)).

Nor did Ms. Distler account for the fact that both agreements include, among other things, “know-how” (Appx3329(209:4-18); Appx3321(172:4-25); Appx9082-9083), even though, as Ms. Distler conceded at trial, a reasonable royalty cannot reflect value for know-how (Appx3327-3328(204:25-205:16)). *See ResQNet.com*, 594 F.3d at 870, 871 (licenses including value beyond the licensed patent rights do not provide a sufficient basis for hypothetical license). To the contrary, applying a royalty value for rights beyond the scope of the hypothetical negotiation

“punishes beyond the reach of the statute.” *ResQNet.com*, 594 F.3d at 869.¹²

Viewed as a whole, both agreements on which Seagen’s damages case depends “are vastly different” in nature, size, and scope from the narrow agreement that Seagen and Appellants would have negotiated for the ’039 Patent. *Lucent*, 580 F.3d at 1328. Indeed, Seagen’s Chief Financial Officer conceded that a “very narrow” license to a single patent was unlike prior Seagen licenses. *See* Appx3337(243:17-24; Appx3337(242:11-18). Such an unexplained mismatch between the broad comparator license and the hypothetical negotiation over only the ’039 Patent provides a legally insufficient factual basis for a royalty award. *See Lucent*, 580 F.3d at 1328 (license to an “entire patent portfolio” was not sufficiently comparable to a negotiation “involving only one patent”); *LaserDynamics, Inc. v. Quanta Computer, Inc.*, 694 F.3d 51, 79 (Fed. Cir.

¹² As a concededly “complex” collaboration agreement (Appx3318(166:12); *see also, e.g.*, Appx9084-9094), the [REDACTED] licensee Agreement is even further afield of the hypothetical negotiation. *See Lucent*, 580 F.3d at 1331 (explaining that testimony is necessary to explain how differences in license “complexity would have affected the hypothetical negotiation analysis”). Indeed, its express terms contradict Ms. Distler’s position. The [REDACTED] licensee Agreement’s royalty rates for Seagen’s ADC technology are well below both Ms. Distler’s proposed rate, and the rates for [REDACTED] licensee patented technology. *See* Appx9110.

2012) (“[T]o prove a reasonable royalty, alleging a loose or vague comparability between different technologies or licenses does not suffice.”).

Ms. Distler proposed that two other agreements—a license information [REDACTED] Agreement (Appx9254-9301), and a 2018 Daiichi Sankyo Agreement (Appx9896-9924)—confirm the reasonableness of her proffered royalty rate (Appx3322(181:8-14)). Those agreements, however, similarly fail to compare with the narrow scope of the hypothetical negotiation in this case—again conferring “exclusive” and “worldwide” rights to multiple patents and patent families. *See* Appx9254; Appx9262; Appx9265; Appx9299; Appx9896; Appx9899; Appx9901; Appx9922.

Because Seagen did not carry its “burden to prove that the licenses relied on were sufficiently comparable to sustain” the jury’s royalty award, *Lucent*, 580 F.3d at 1332, the district court’s refusal to grant JMOL or a new trial on the basis of that award cannot stand. *See id.*; *Wordtech*, 609 F.3d at 1322 (reversing and remanding for a new trial where damages award was “clearly not supported by the evidence and based on speculation and guesswork” (citation and internal quotation marks omitted)).

B. Appellants' challenge is properly before this Court.

In both Rule 50(a) and Rule 50(b) motions, Appellants challenged the legal sufficiency of Seagen's damages evidence based on, *inter alia*, the materially different scope of the hypothetical negotiation and the agreements relied on by Ms. Distler. See Appx1848-1850; Appx3356(318:4-8); Appx2191-2196. Nothing more is required to preserve a challenge for appeal. See, e.g., *Colonial Penn. Ins. v. Market Planners Ins. Agency Inc.*, 157 F.3d 1032, 1036 n.3 (5th Cir. 1998).

In denying Appellants' Rule 50(b) motion, the district court first concluded—with no elaboration—that “reasonable evidence” supported both Seagen's and Appellants' damages positions, and the jury was entitled to weigh the evidence and choose Seagen's position. Appx40. Nevertheless, the district court went on to note its agreement with Seagen's argument that Appellants' Rule 50(b) motion was “akin to a post-trial *Daubert* motion” and that such a delayed attack on “an expert's methodology” is improper. Appx40. The district court's reference to

Daubert confuses the admissibility of Seagen’s expert testimony (which Appellants did not challenge), with the sufficiency of Seagen’s evidence.¹³

Admissibility and sufficiency are separate inquiries, and a reviewing court assesses the legal sufficiency of the evidence supporting damages even if there is no question as to the admissibility of either the testimony of the patentee’s damages expert or the licenses on which the expert relied. *Lucent*, 580 F.3d at 1325. Indeed, in *Lucent Technologies*, this Court concluded that the license agreements underlying a damages verdict were not sufficiently comparable to support a damages award even though the defendant had waived any challenge to the admissibility of the licenses or to the testimony of the plaintiff’s expert. *Id.* (“[W]e must accept that the licensing agreements and other evidence were properly before the jury.”); *cf. ResQNet.com*, 594 F.3d at 871 (characterizing reliance on licenses that are not comparable to hypothetical negotiation as “legal error”). The same inquiry and result apply here.

¹³ Appellants also moved for a new trial on damages, arguing that (1) the Court erred in excluding an instruction on evaluating comparable patent licenses, and (2) the damages award was excessive because based on licenses that are not comparable to the hypothetical negotiation. Appx2221; Appx2224. The district court concluded that its instructions were proper, and denied the new trial motion for the same reasons it denied the Rule 50(b) motion. Appx2457-2458.

CONCLUSION

For the foregoing reasons, the Court should reverse the judgment below or vacate and remand with respect to the damages verdict.

March 22, 2024

Respectfully submitted,

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ADDENDUM

ADDENDUM CONTENTS

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**IN THE UNITED STATES DISTRICT COURT
FOR THE EASTERN DISTRICT OF TEXAS
MARSHALL DIVISION**

SEAGEN INC.,	§	
	§	
<i>Plaintiff,</i>	§	
	§	
v.	§	CIVIL ACTION NO. 2:20-CV-00337-JRG
	§	
DAIICHI SANKYO CO., LTD.,	§	
	§	
<i>Defendant,</i>	§	
	§	
ASTRAZENECA PHARMACEUTICALS	§	
LP, and ASTRAZENECA UK LTD	§	
	§	
<i>Intervenor-Defendants.</i>	§	

AMENDED¹ FINAL JUDGMENT

A jury trial commenced in the above-captioned case on April 4, 2022, and on April 8, 2022, the jury reached and returned its unanimous verdict finding that Defendant Daiichi Sankyo Company, Limited (“DSC”) infringed at least one of Claims 1–5, 9, and 10 of U.S. Patent No. 10,808,039 (the “’039 Patent”) (collectively, the “Asserted Claims”); that such infringement was willful; that none of the Asserted Claims were invalid; and that Plaintiff Seagen, Inc. (“Seagen”) is owed a reasonable royalty of \$41,820,000.00 for DSC’s infringement from October 20, 2020 through March 31, 2022. (Dkt. No. 370).

The Court conducted a separate bench trial on June 28, 2022 regarding DSC’s prosecution laches and § 112(b) defenses. On July 15, 2022, the Court issued Findings of Fact and Conclusions of Law, wherein the Court held that DSC had not proven by clear and convincing evidence that

¹ The Court enters this Amended Final Judgment to include the relief granted in Dkt. No. 496, as clarified in the amended version of that order (Dkt. No. 511), to the Final Judgment entered by the Court on July 19, 2022 (Dkt. No. 432). This Amended Final Judgment supersedes the prior Final Judgment (Dkt. No. 432) and resolves all issues before the Court.

the '039 Patent was unenforceable due to prosecution laches or invalid under § 112(b). (Dkt. No. 431).

Thereafter, Seagen filed a Motion for Judgment for Supplemental Damages and Ongoing Royalties (the “Damages Motion”). (Dkt. No. 443). On August 21, 2023, the Court granted in part Seagen’s Damages Motion and found that supplemental damages and an ongoing royalty award were appropriate. (Dkt. No. 496). Specifically, the Court awarded Seagen supplemental damages and an ongoing royalty from DSC at the rate of 8% for U.S. sales of Enhertu by DSC’s wholly owned subsidiary Daiichi Sankyo, Inc. (“DSI”) for the period from April 1, 2022 (the commencement of trial) through November 4, 2024 (the end of the '039 Patent’s life). (*Id.* at 14). The Court also ordered that DSC provide Seagen with an accounting of the sales by DSI of Enhertu in the United States from April 1, 2022 through the date of that order (August 21, 2023). (*Id.*). The Court further ordered DSC to provide Seagen with an update of DSI’s sales of Enhertu in the United States every three months from August 21, 2023 through November 4, 2024. (*Id.*).

Pursuant to Rule 58 of the Federal Rules of Civil Procedure, and in accordance with the jury’s unanimous verdict and the entirety of the record, the Court hereby **ORDERS** and **ENTERS JUDGMENT** as follows:

1. DSC has infringed at least one of the Asserted Claims;
2. The Asserted Claims are not invalid;
3. DSC’s infringement was willful;
4. Seagen is hereby awarded damages from and against DSC and shall accordingly have and recover from DSC the sum of \$41,820,000.00 U.S. Dollars as a reasonable royalty for sales from October 20, 2020 through March 31, 2022;

5. Seagen is hereby awarded supplemental damages and an ongoing royalty at the rate of 8% from and against DSC for the sales of Enhertu in the United States by DSI for the period from April 1, 2022 through November 4, 2024, with such payments to be made no less frequently than quarterly;
6. DSC shall provide Seagen with an accounting of the sales of Enhertu in the United States by DSI for the period from April 1, 2022 through August 21, 2023;
7. DSC shall provide Seagen with an update of DSI's sales of Enhertu in the United States every three months for the period from August 21, 2023 through November 4, 2024;
8. Notwithstanding the jury's finding of willfulness, the Court having considered the totality of the circumstances together with the added material benefit of having presided throughout the jury trial and having seen both the same evidence and heard the same arguments as the jury, and mindful that enhancement is generally reserved for "egregious cases of culpable behavior,"² concludes that enhancement of the compensatory award herein is not warranted under 35 U.S.C. § 284 and consequently, the Court elects not to enhance the damages awarded herein;
9. Pursuant to 35 U.S.C. § 284 and Supreme Court guidance that "prejudgment interest shall ordinarily be awarded absent some justification for withholding such an award,"³ the Court awards pre-judgment interest applicable to all sums awarded herein, calculated at the 5-year U.S. Treasury Bill rate, compounded quarterly, from the date of infringement through the date of entry of this Judgment;⁴ and

² *Halo Electronics, Inc. v. Pulse Electronics, Inc.*, 136 S.Ct. 1923, 1934 (2016).

³ *General Motors Corp. v. Devex Corp.*, 461 U.S. 648, 657 (1983).

⁴ *See Nickson Indus., Inc. v. Rol Mfg. Co., Ltd.*, 847 F.2d 795, 800–801 (Fed. Cir. 1988).

10. Pursuant to 28 U.S.C. § 1961, the Court awards post-judgment interest applicable to all sums awarded herein, at the statutory rate, from the date of entry of this Judgment until paid.

11. Pursuant to Federal Rule of Civil Procedure 54(d), Local Rule CV-54, and 28 U.S.C. § 1920, Seagen is the prevailing party in this case and shall recover its costs from DSC. Seagen is directed to file its proposed Bill of Costs.

All other requests for relief now pending and requested by either party but not specifically addressed herein are **DENIED**.

So ORDERED and SIGNED this 17th day of October, 2023.



RODNEY GILSTRAP
UNITED STATES DISTRICT JUDGE

**IN THE UNITED STATES DISTRICT COURT
FOR THE EASTERN DISTRICT OF TEXAS
MARSHALL DIVISION**

SEAGEN INC.,

Plaintiff,

V.

DAIICHI SANKYO CO., LTD.,

Defendant,

ASTRAZENECA PHARMACEUTICALS
LP, and ASTRAZENECA UK LTD

Intervenor-Defendants.

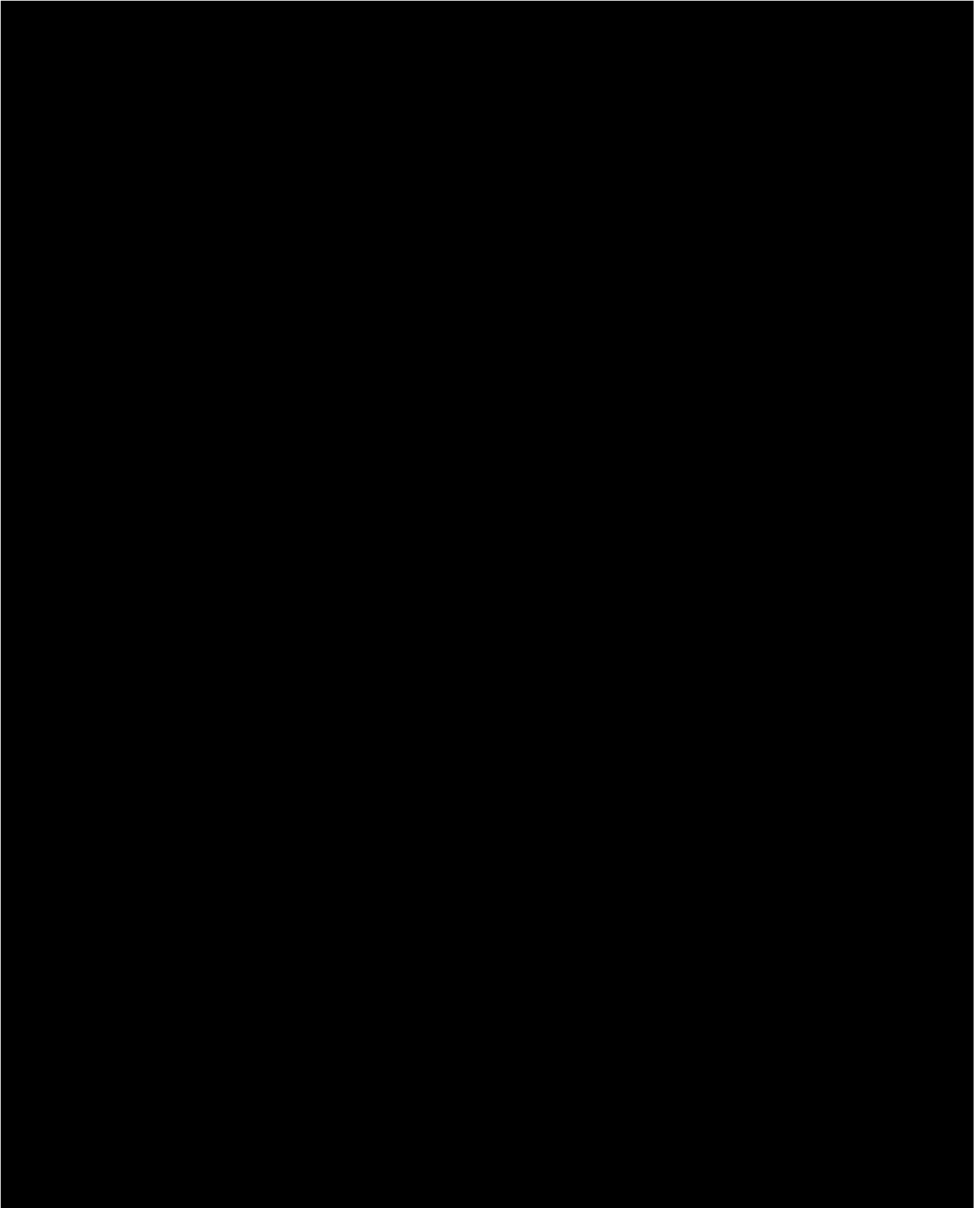
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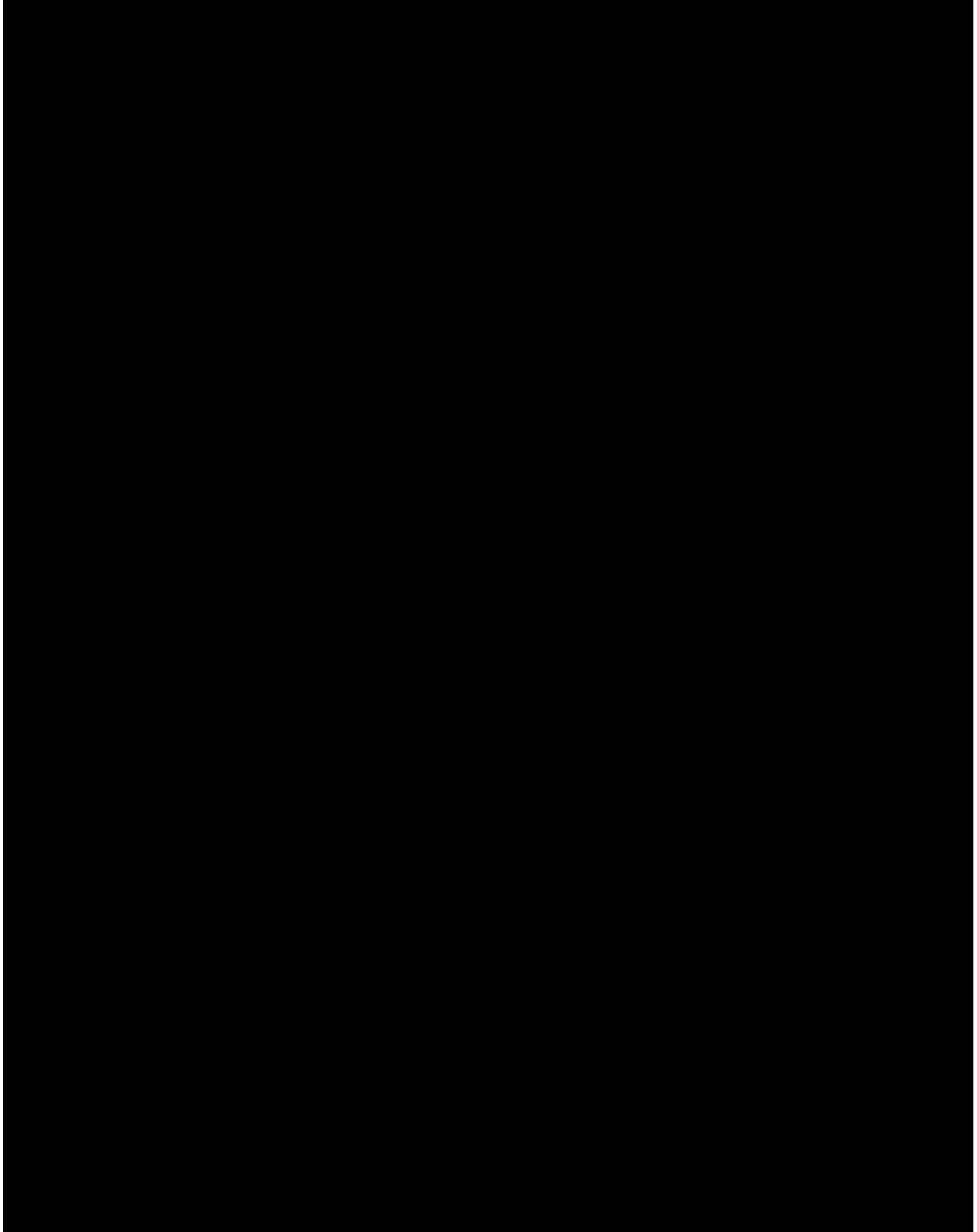
CIVIL ACTION NO. 2:20-CV-00337-JRG

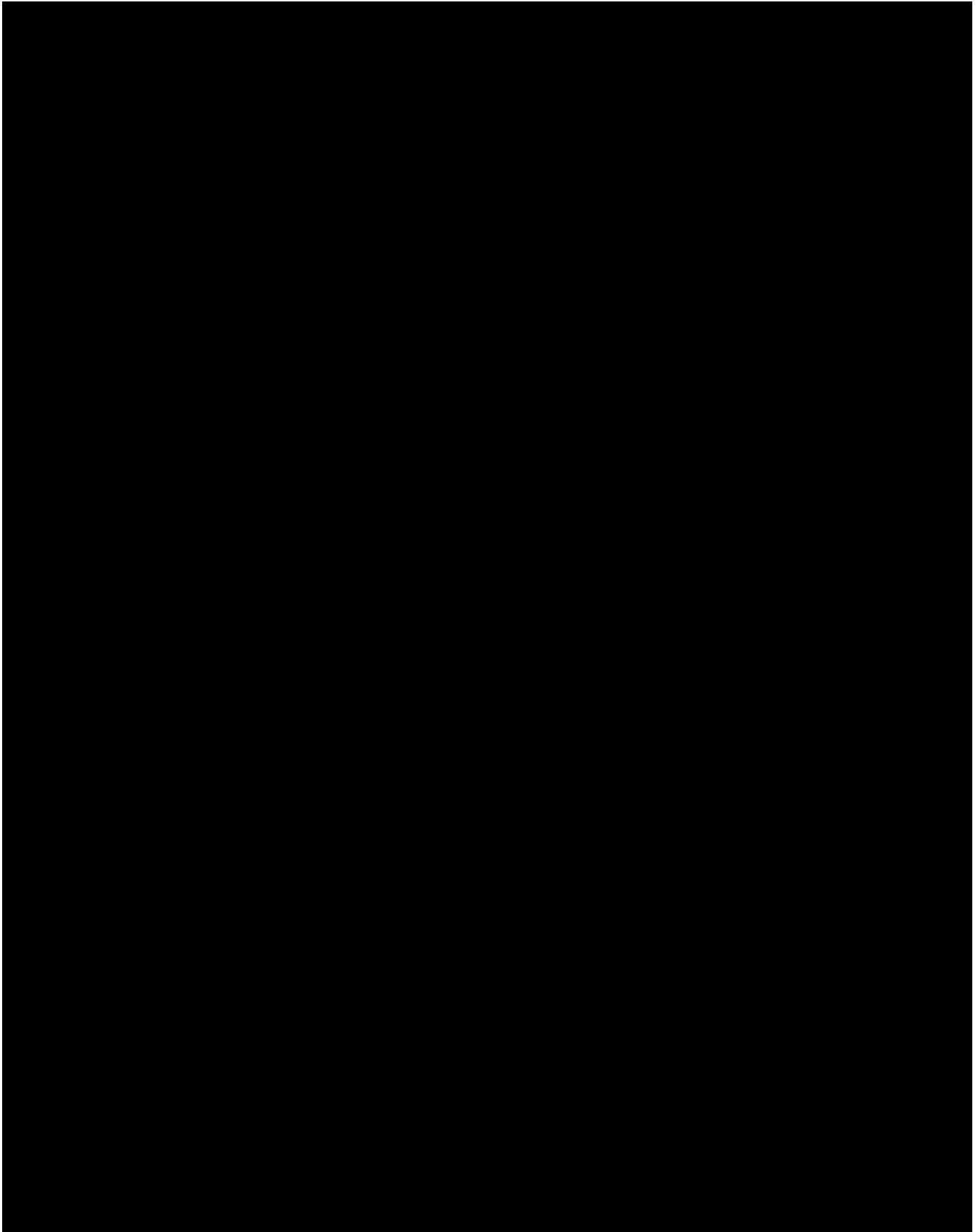
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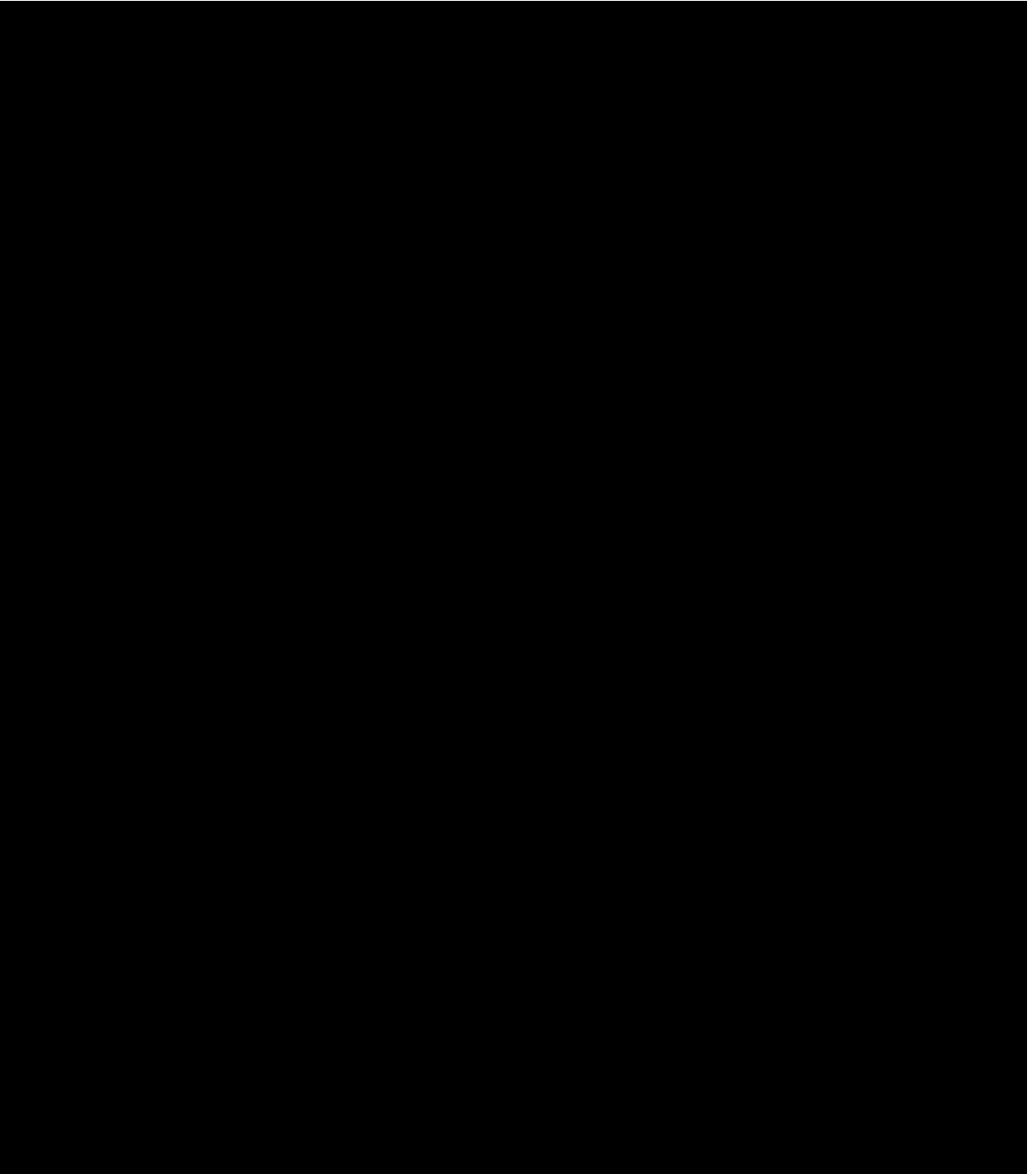
AMENDED¹ MEMORANDUM OPINION AND ORDER

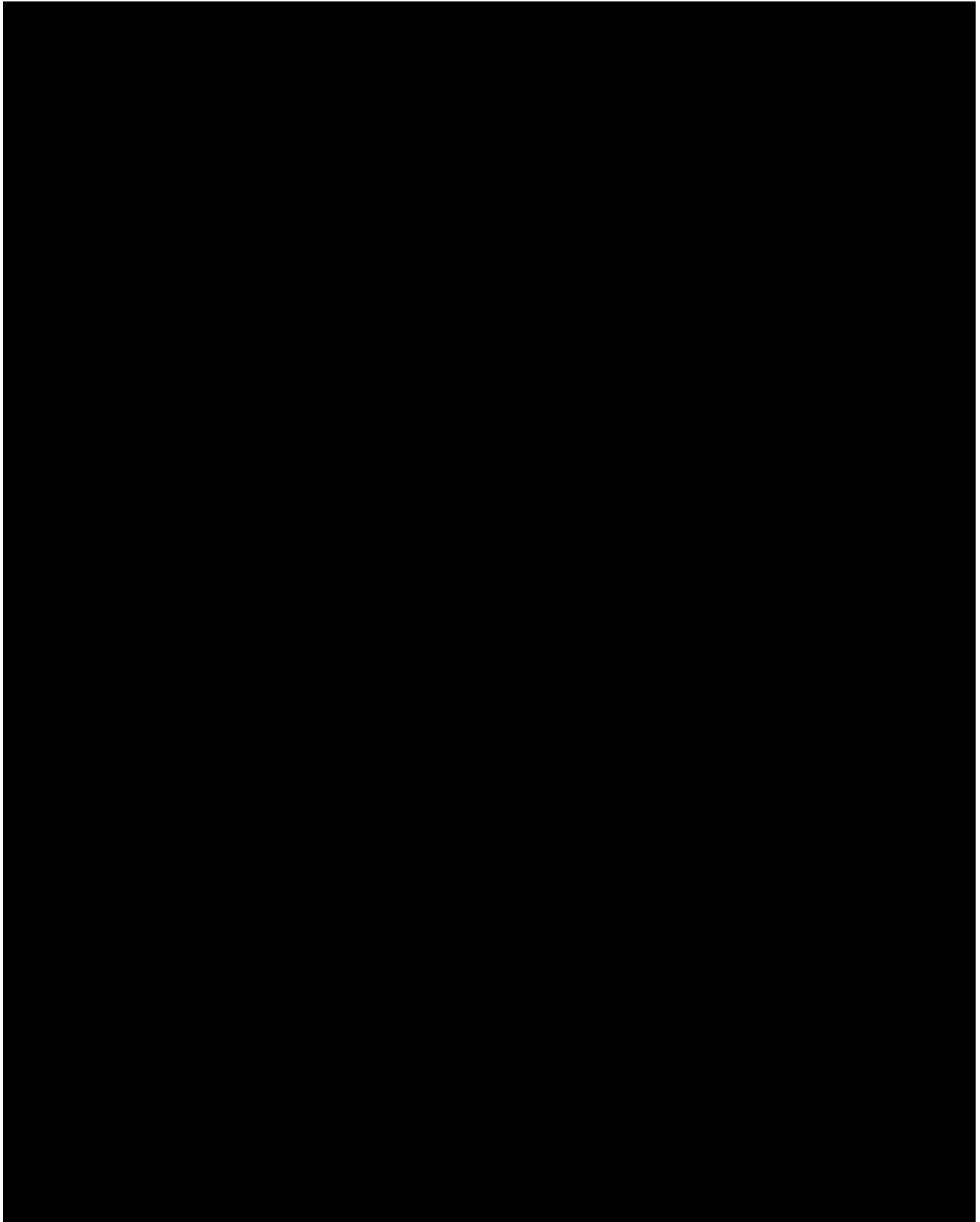
Before the Court are Plaintiff Seagen Inc.’s (“Plaintiff”) Motion for Judgment for Supplemental Damages and Ongoing Royalties (the “Damages Motion”) (Dkt. No. 443) and Motion for an Exceptional Case Finding and Attorney Fees Under 35 U.S.C. § 285 (the “Fees Motion”) (Dkt. No. 435) (collectively, “Motions”). Having considered the Motions and the related briefing, the Court is of the opinion that the Fees Motion should be **DENIED** and the Damages Motion should be **GRANTED-IN-PART**.

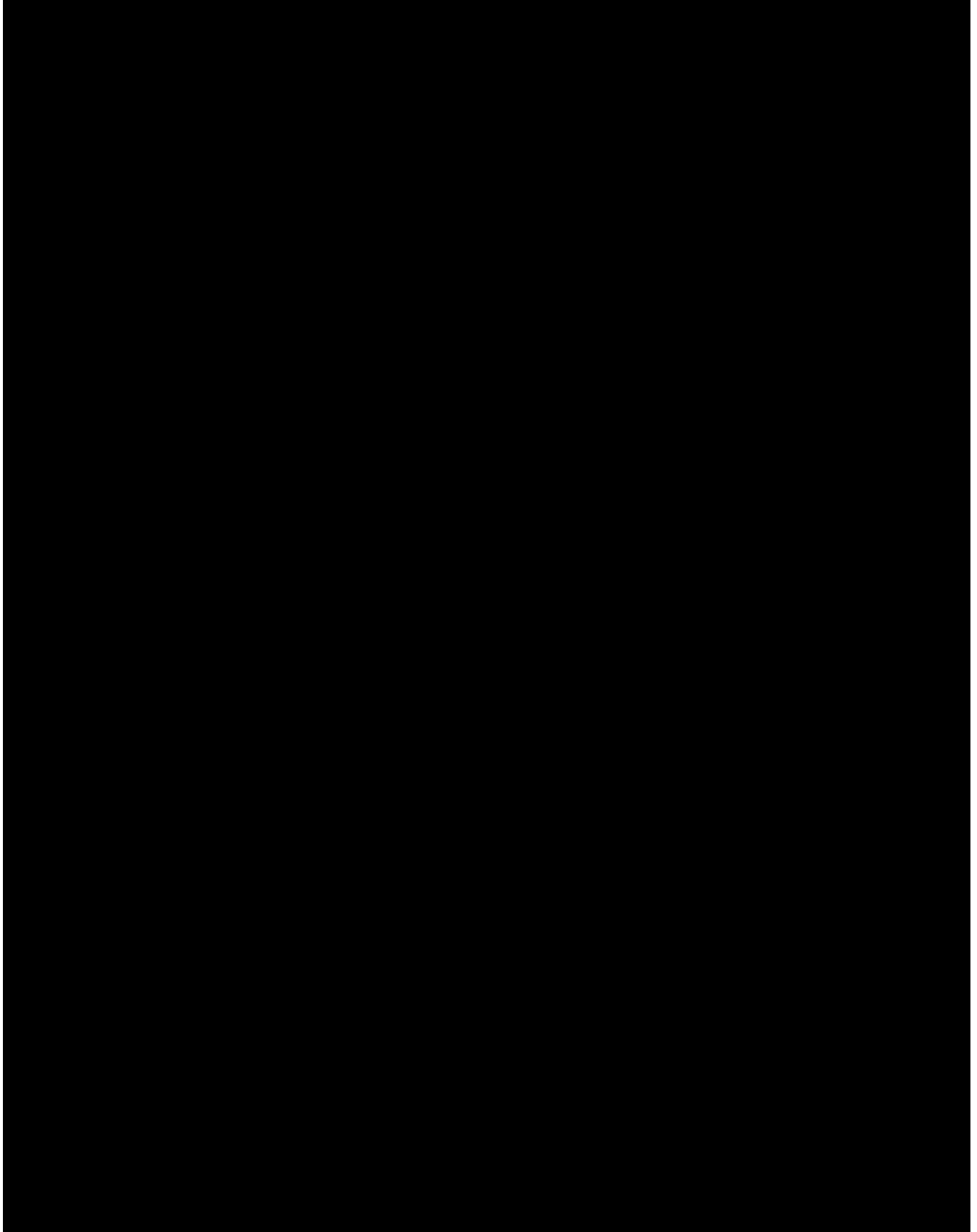


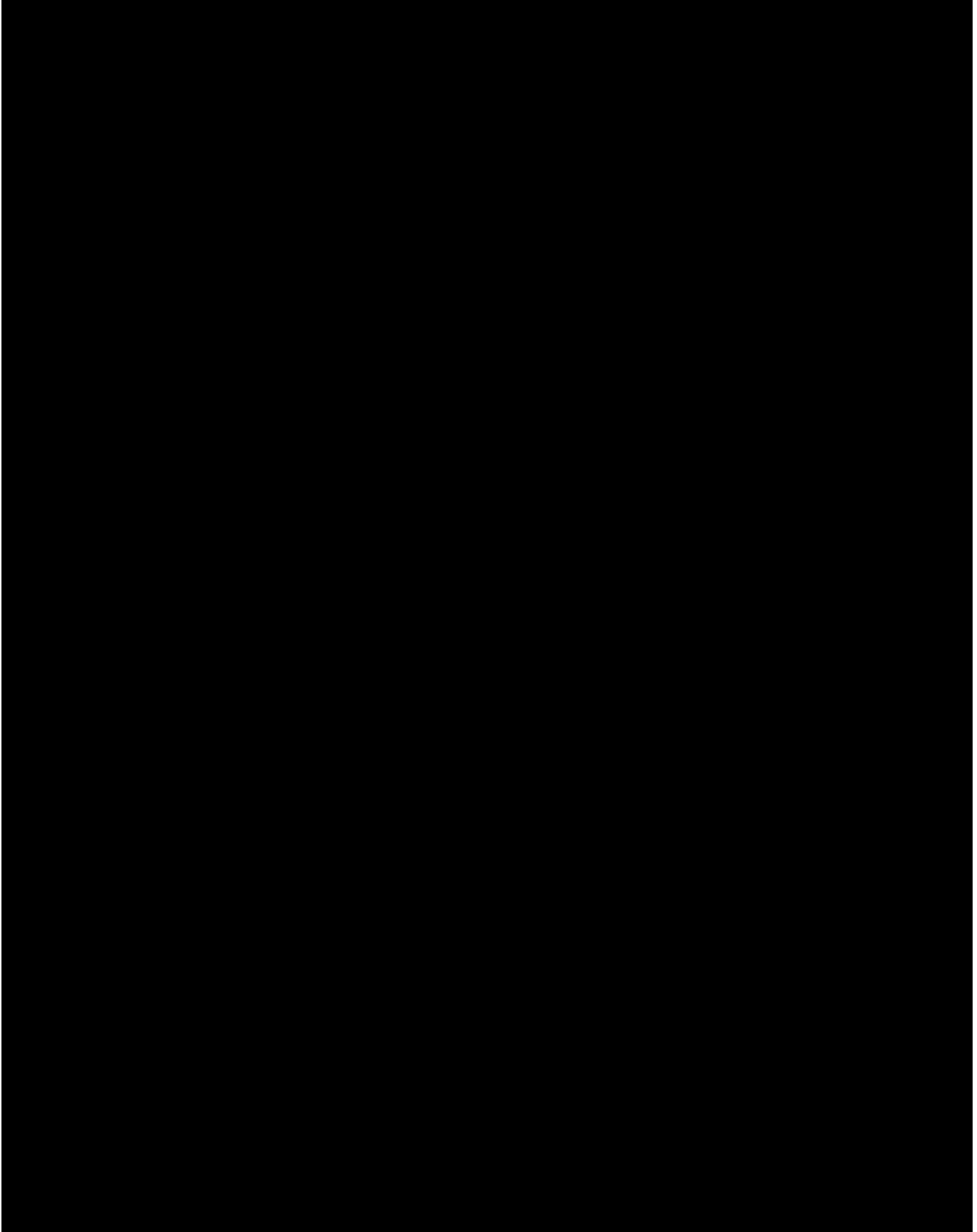


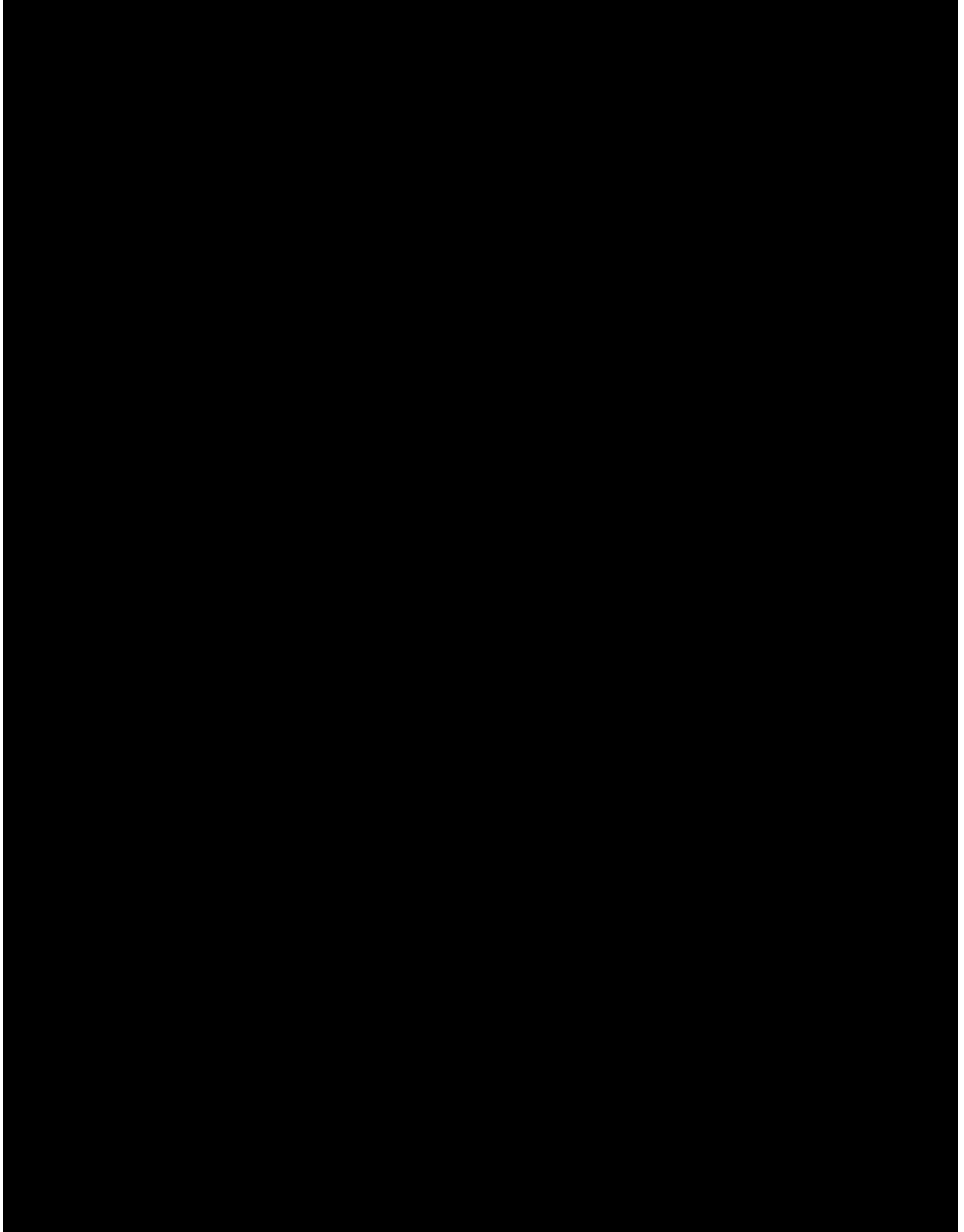


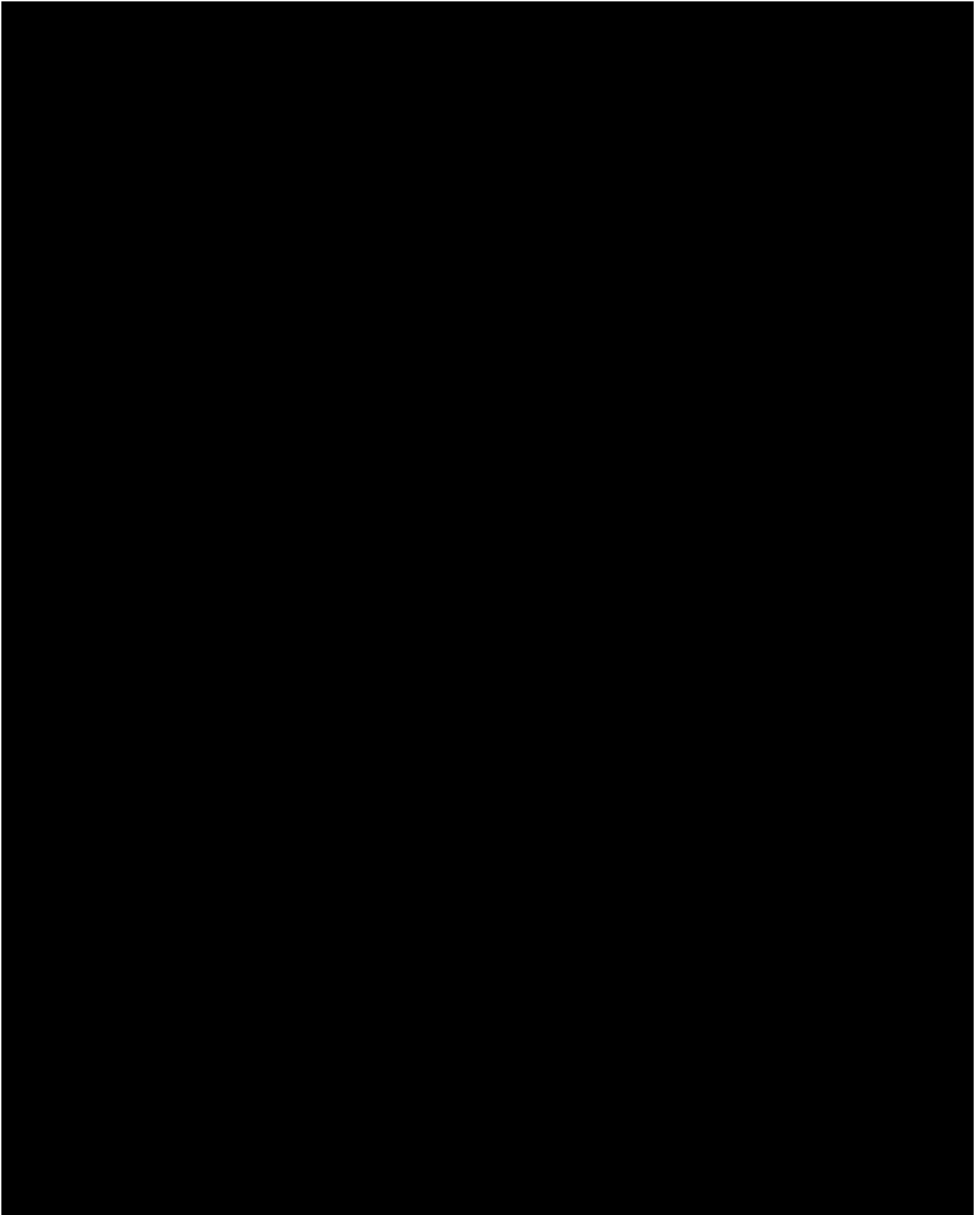


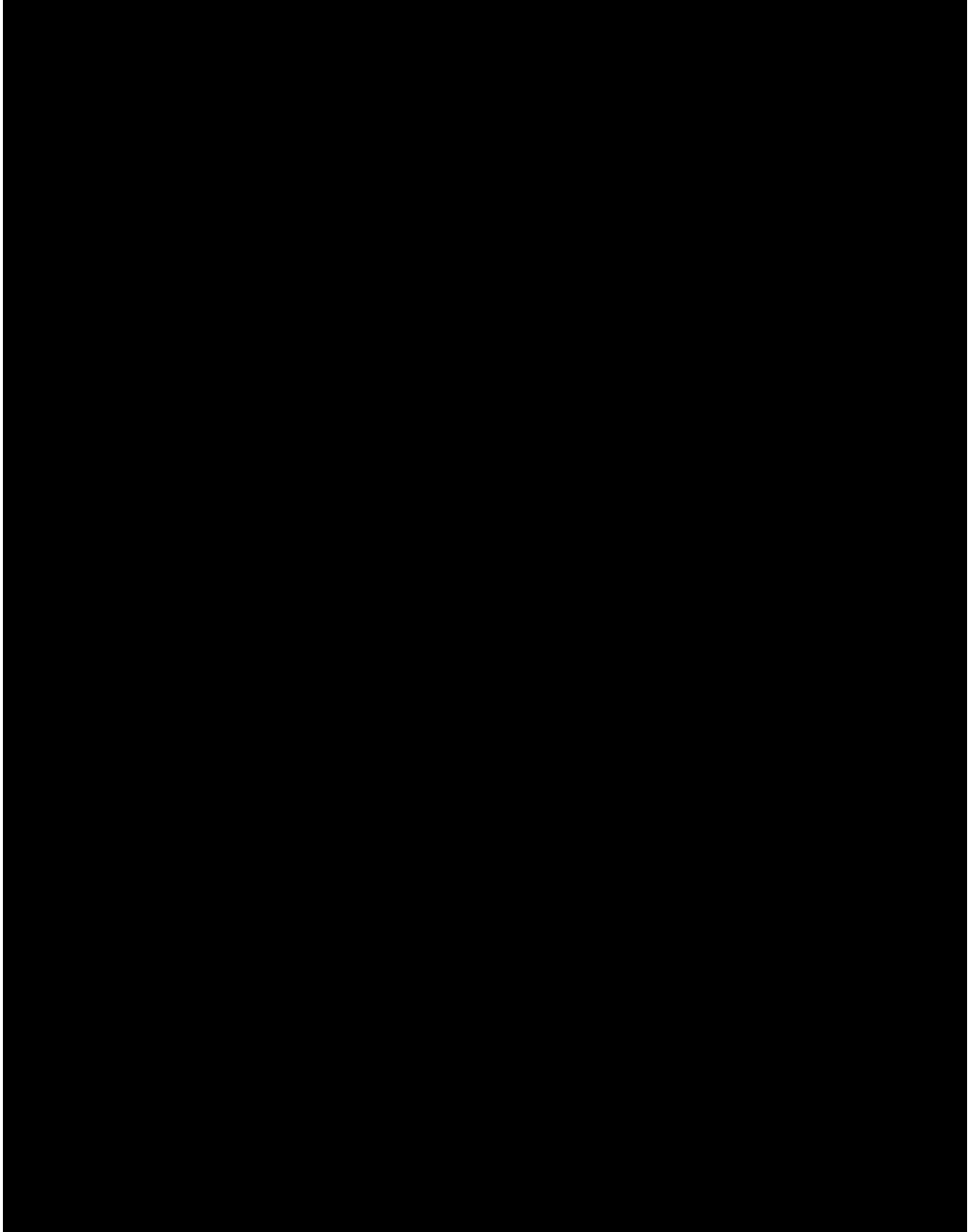


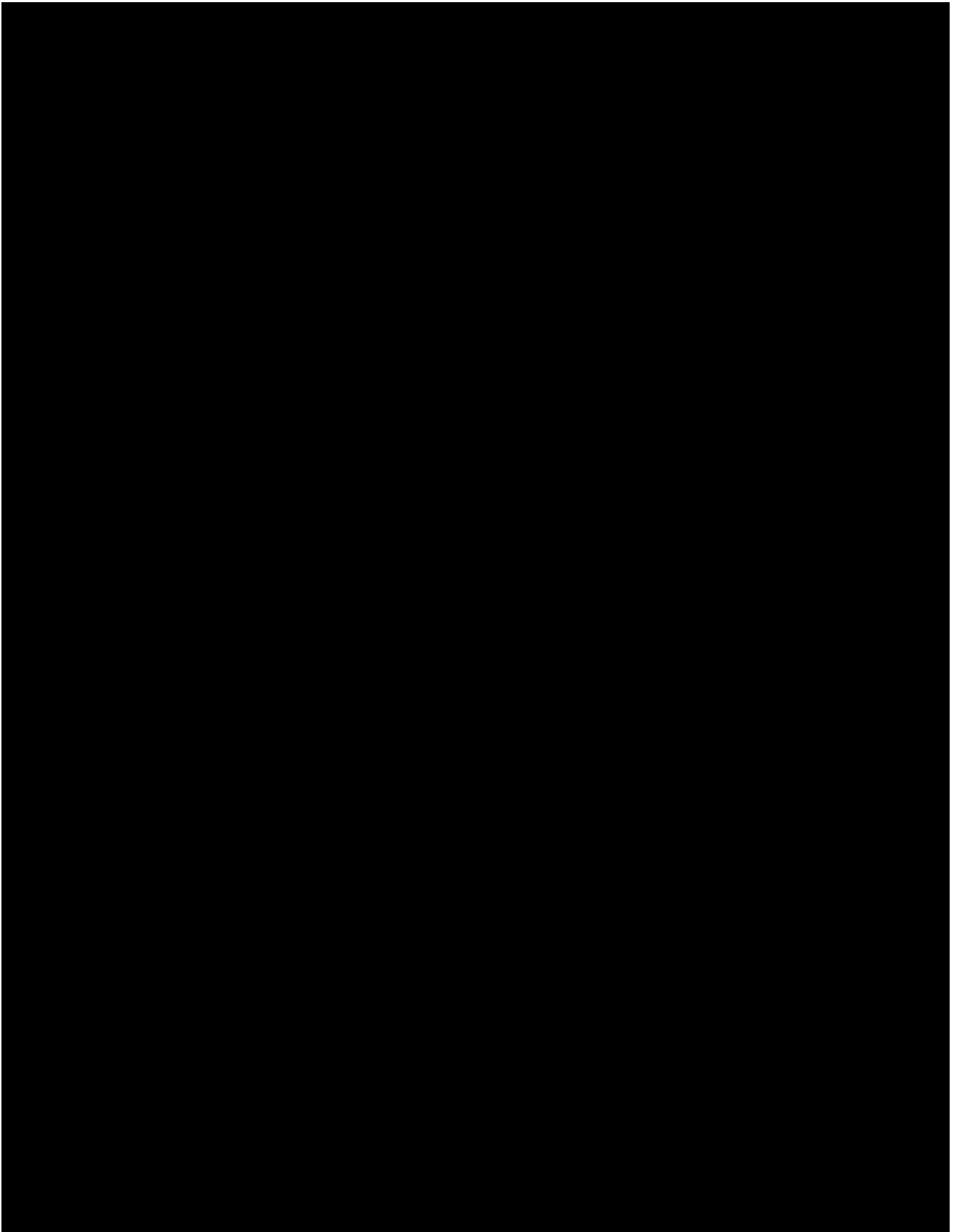


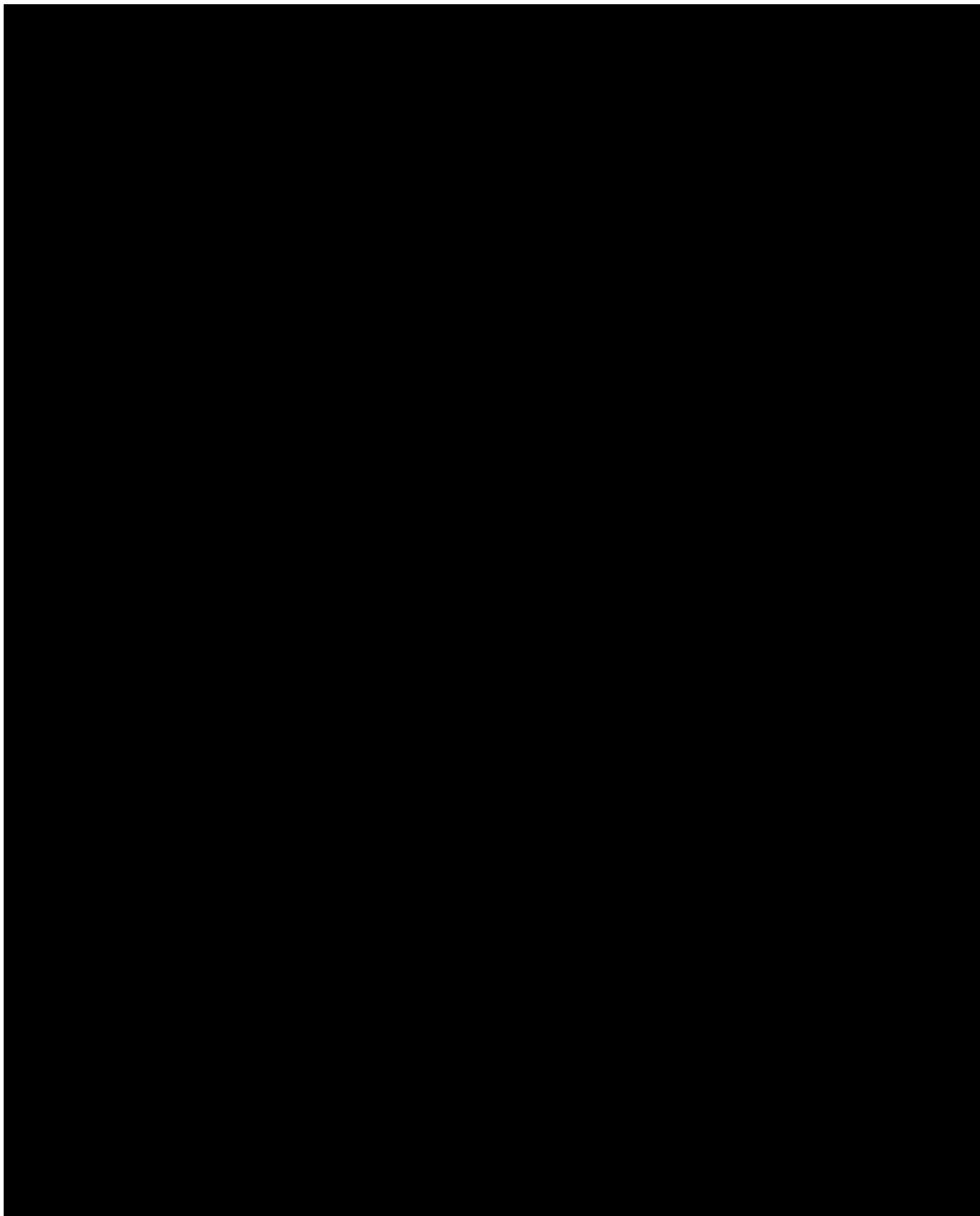


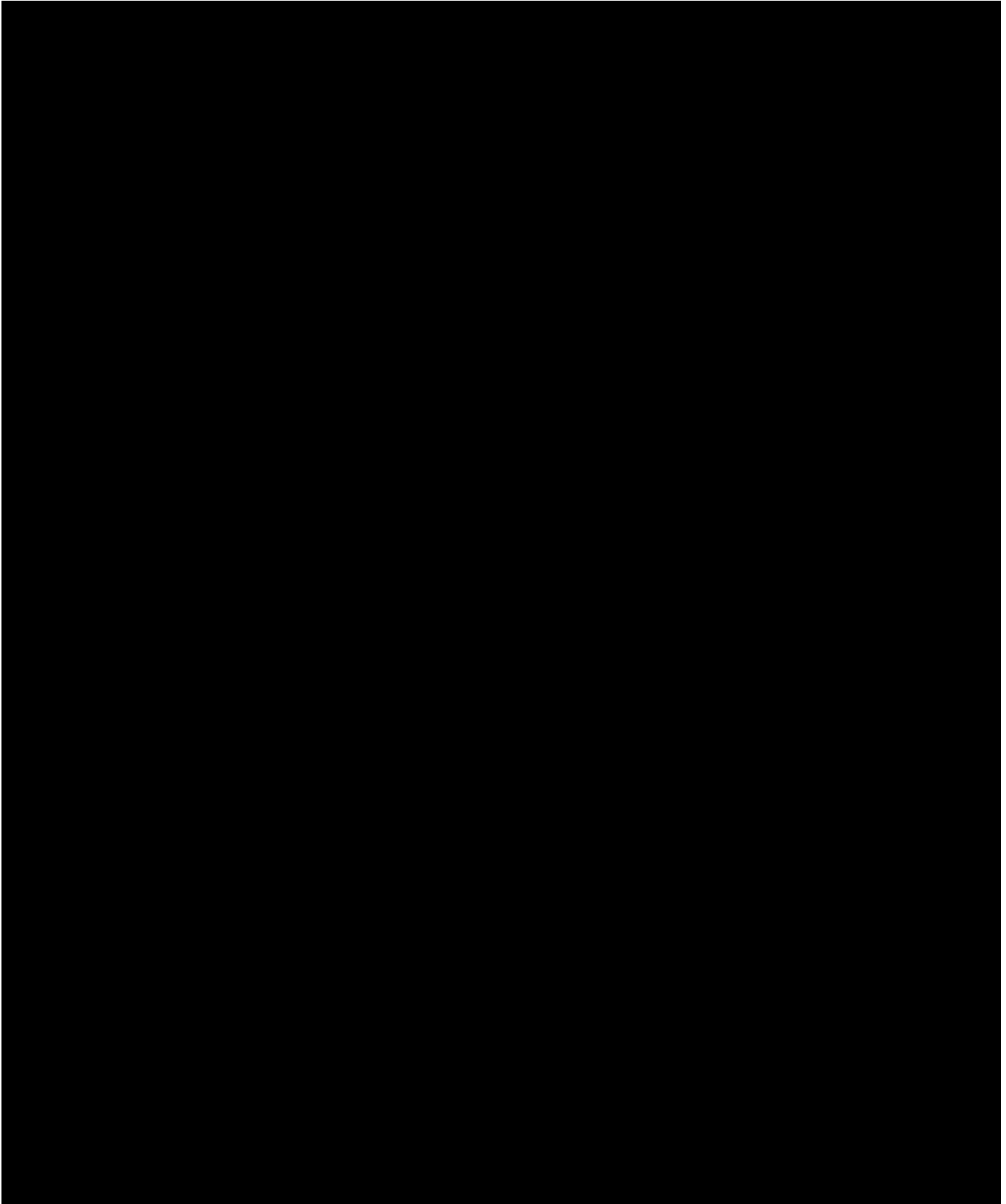













IV. CONCLUSION

For the reasons set forth herein, Plaintiff's Motion for an Exceptional Case Finding and Attorney Fees Under 35 U.S.C. § 285 (Dkt. No. 435) is **DENIED** and Motion for Judgment for Supplemental Damages and Ongoing Royalties (Dkt. No. 443) is **GRANTED-IN-PART**.

So **ORDERED** and **SIGNED** this 17th day of October, 2023.



RODNEY GILSTRAP
UNITED STATES DISTRICT JUDGE

IN THE UNITED STATES DISTRICT COURT
FOR THE EASTERN DISTRICT OF TEXAS
MARSHALL DIVISION

SEAGEN INC.,

Plaintiff,

v.

DAIICHI SANKYO CO., LTD.,

Defendant,

ASTRAZENECA PHARMACEUTICALS
LP, and ASTRAZENECA UK LTD

Intervenor-Defendants.

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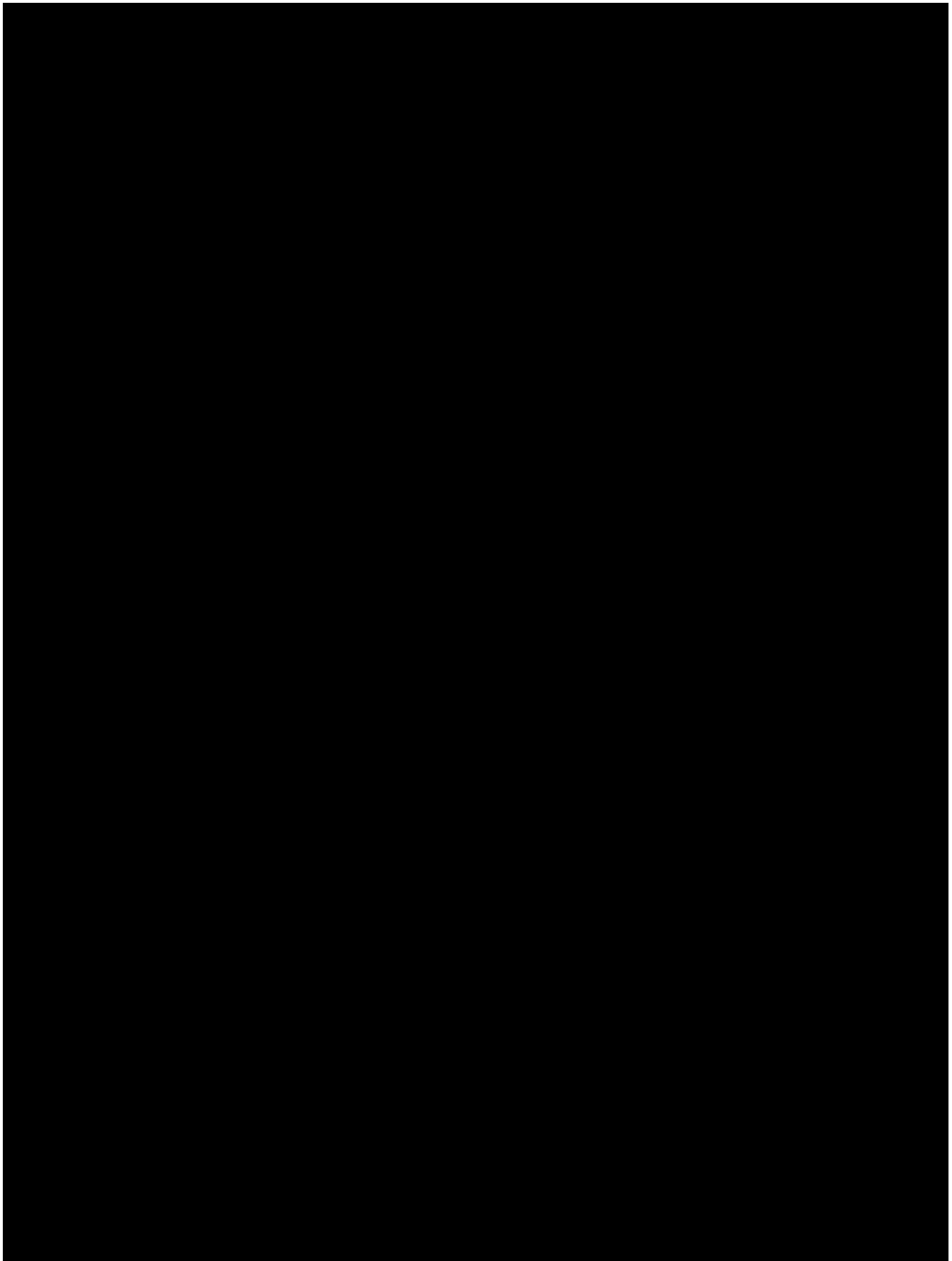
CIVIL ACTION NO. 2:20-CV-00337-JRG

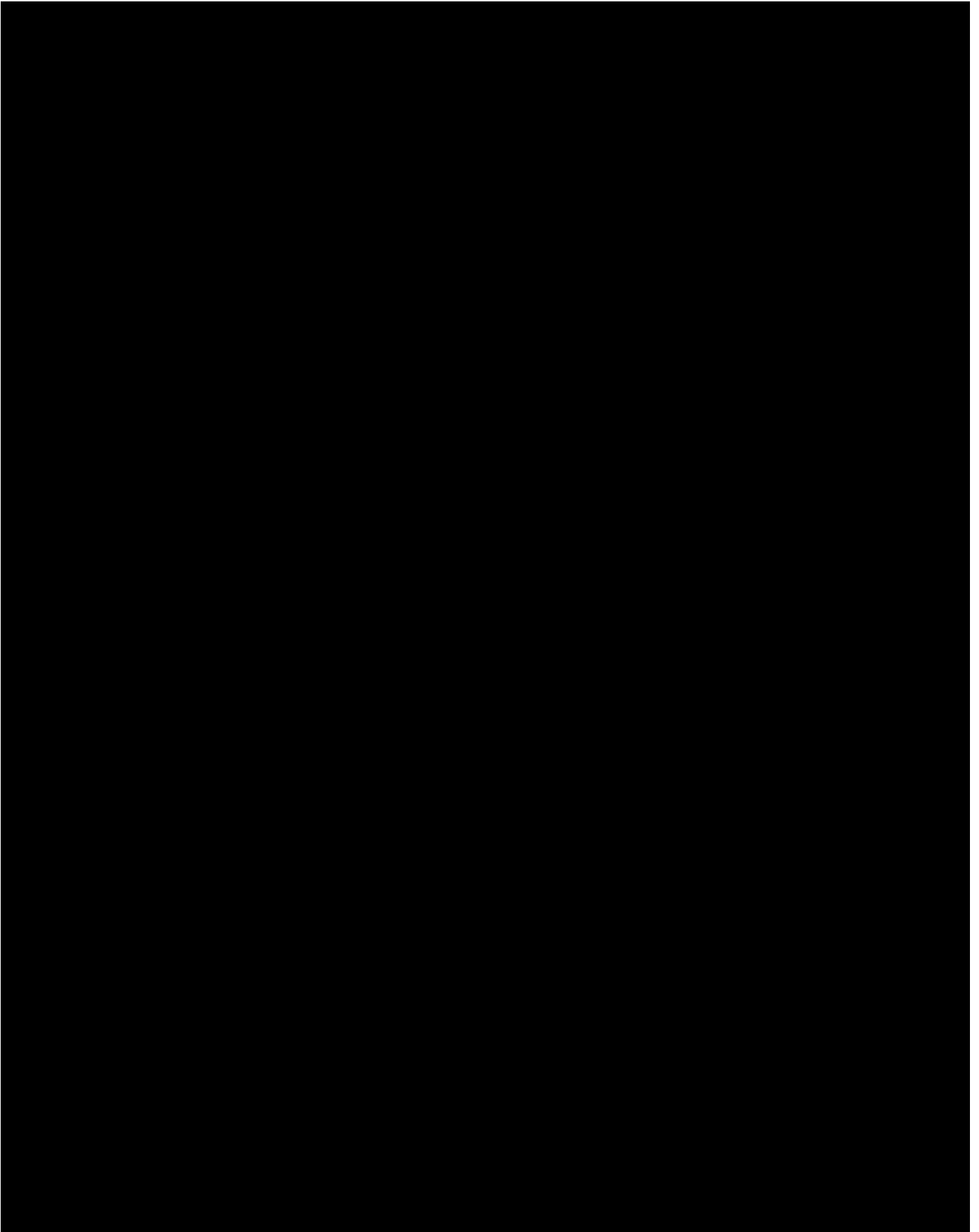
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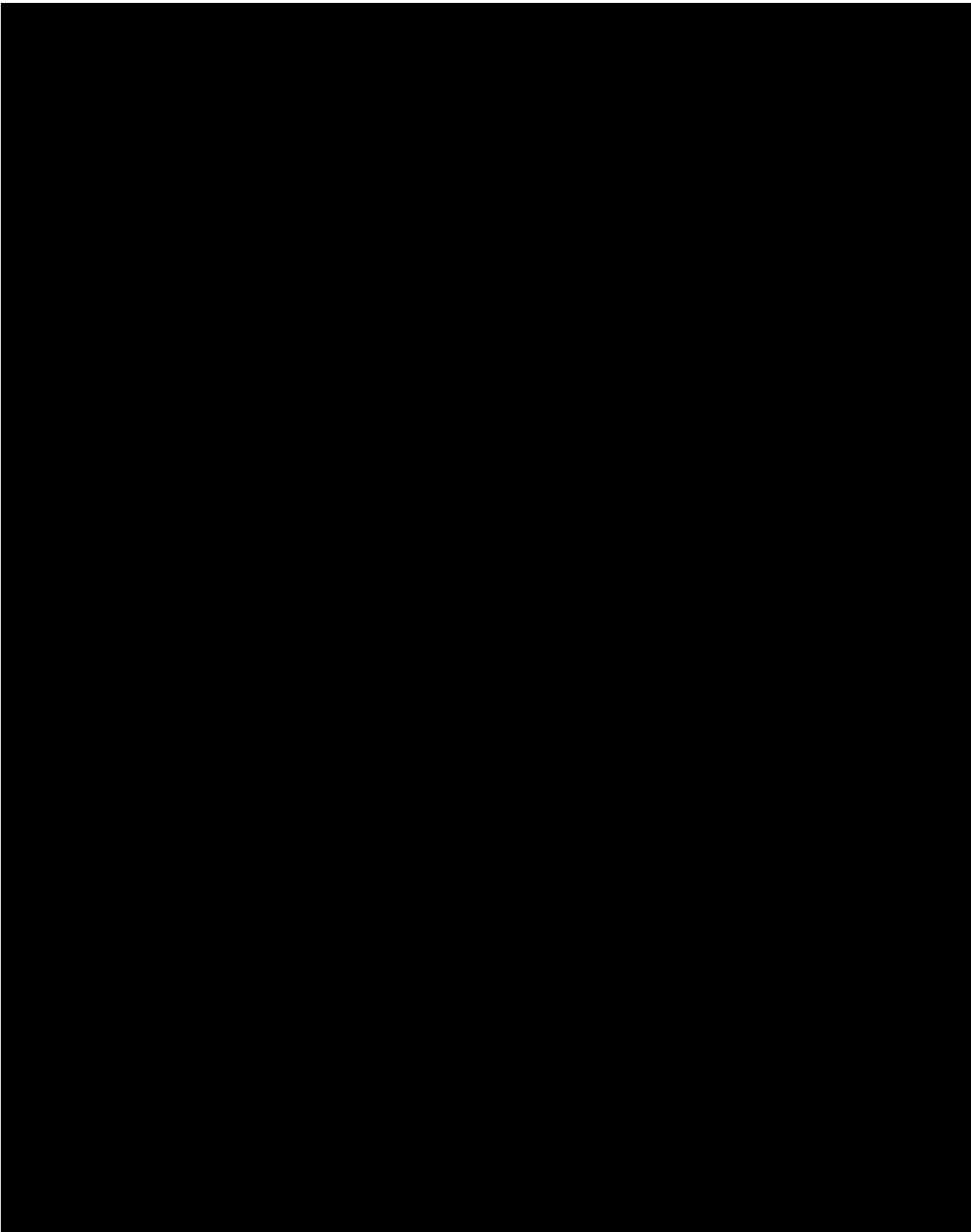
ORDER

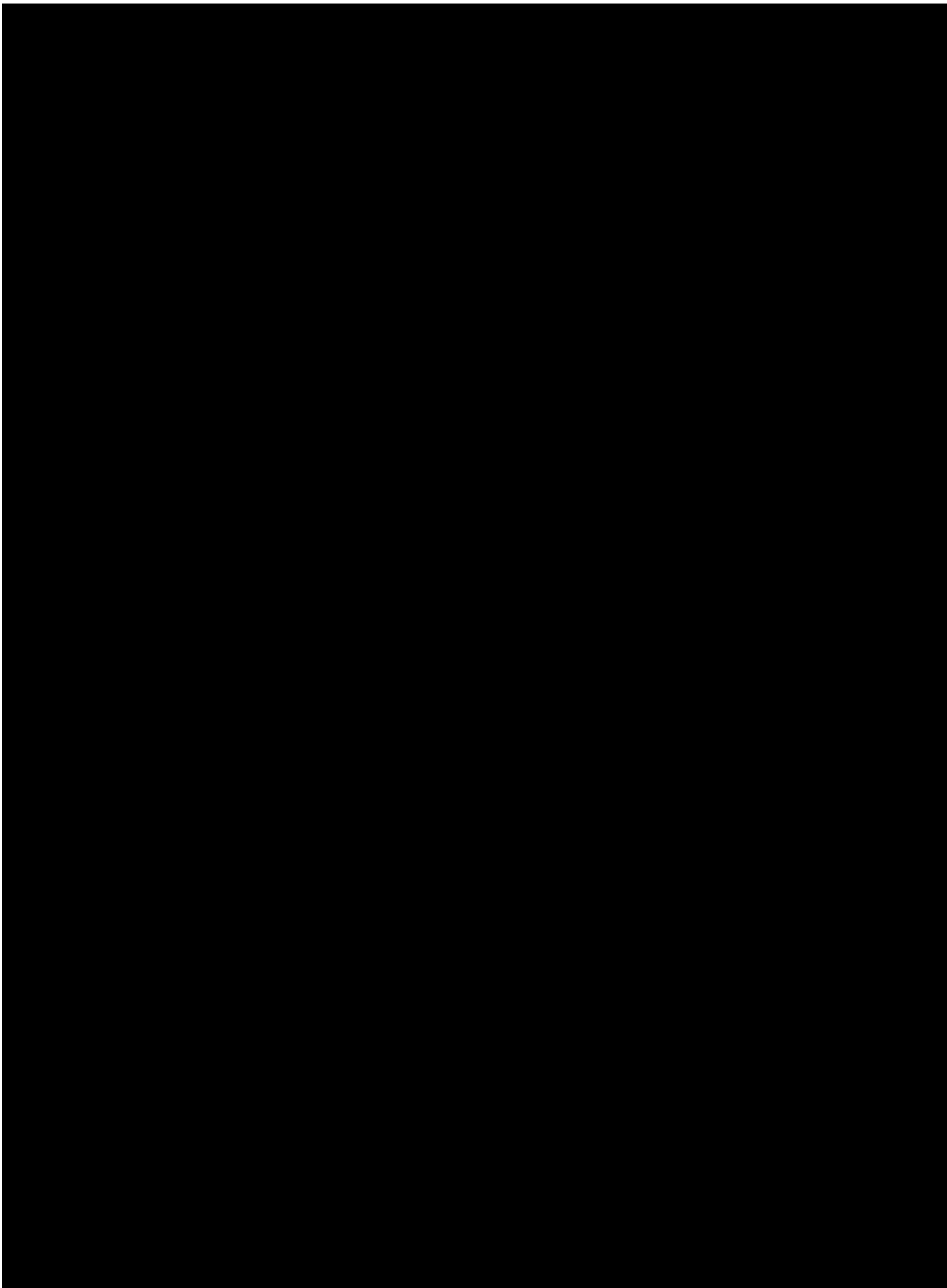
I. INTRODUCTION

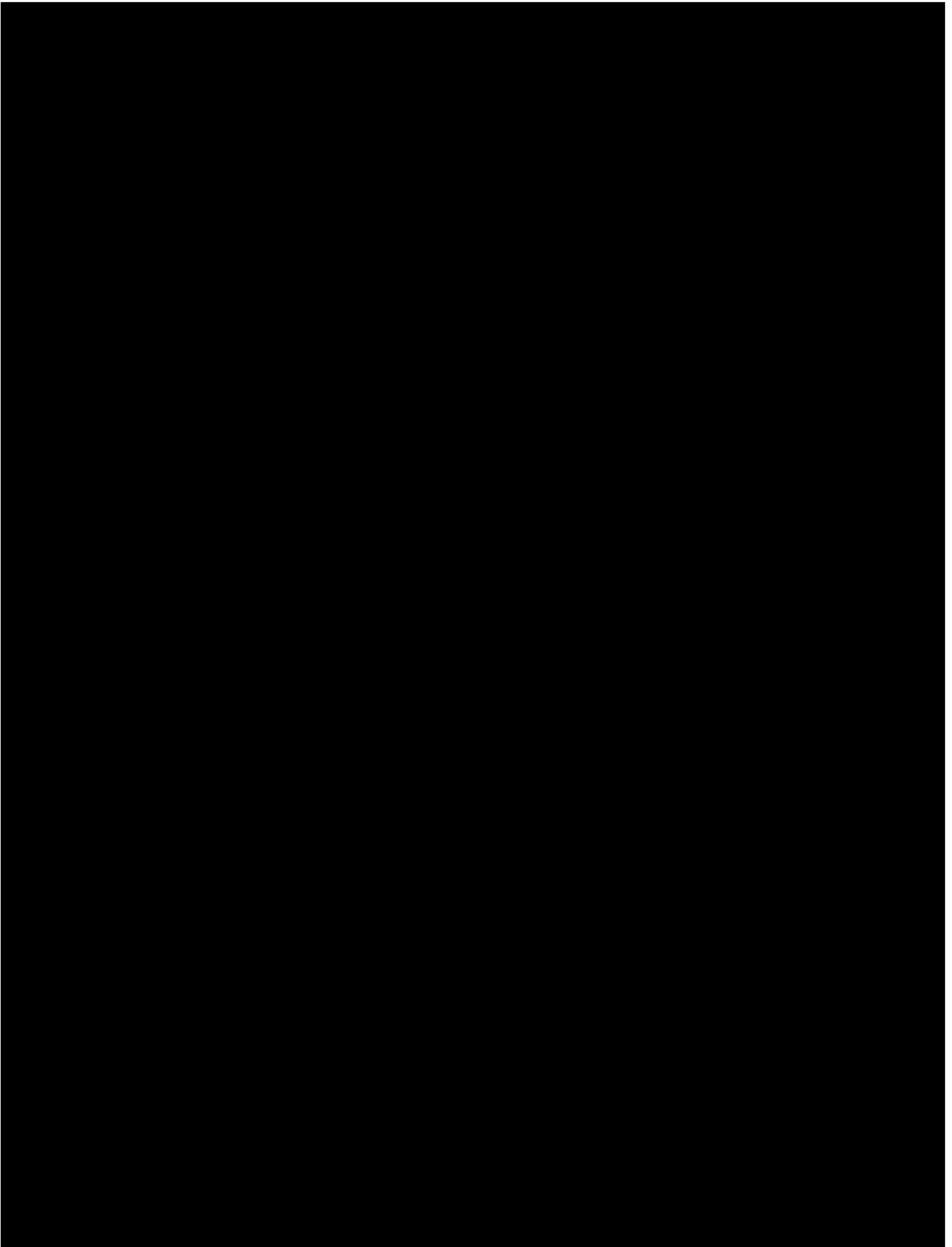
Before the Court is the Opposed Motion Under Federal Rules of Civil Procedure 52(b) and 59(e) to Amend or Add Findings of Fact and Alter or Amend the Judgment (the “Motion to Amend”) filed by Defendant Daiichi Sankyo Company, Limited (“DSC”) and Intervenor-Defendants AstraZeneca Pharmaceuticals LP and AstraZeneca UK Ltd (“AstraZeneca”) (collectively, “Defendants”). (Dkt. No. 499). Having considered the Motion to Amend, related briefing, and the applicable law, the Court finds that the Motion to Amend should be **GRANTED IN PART** and **DENIED IN PART**.

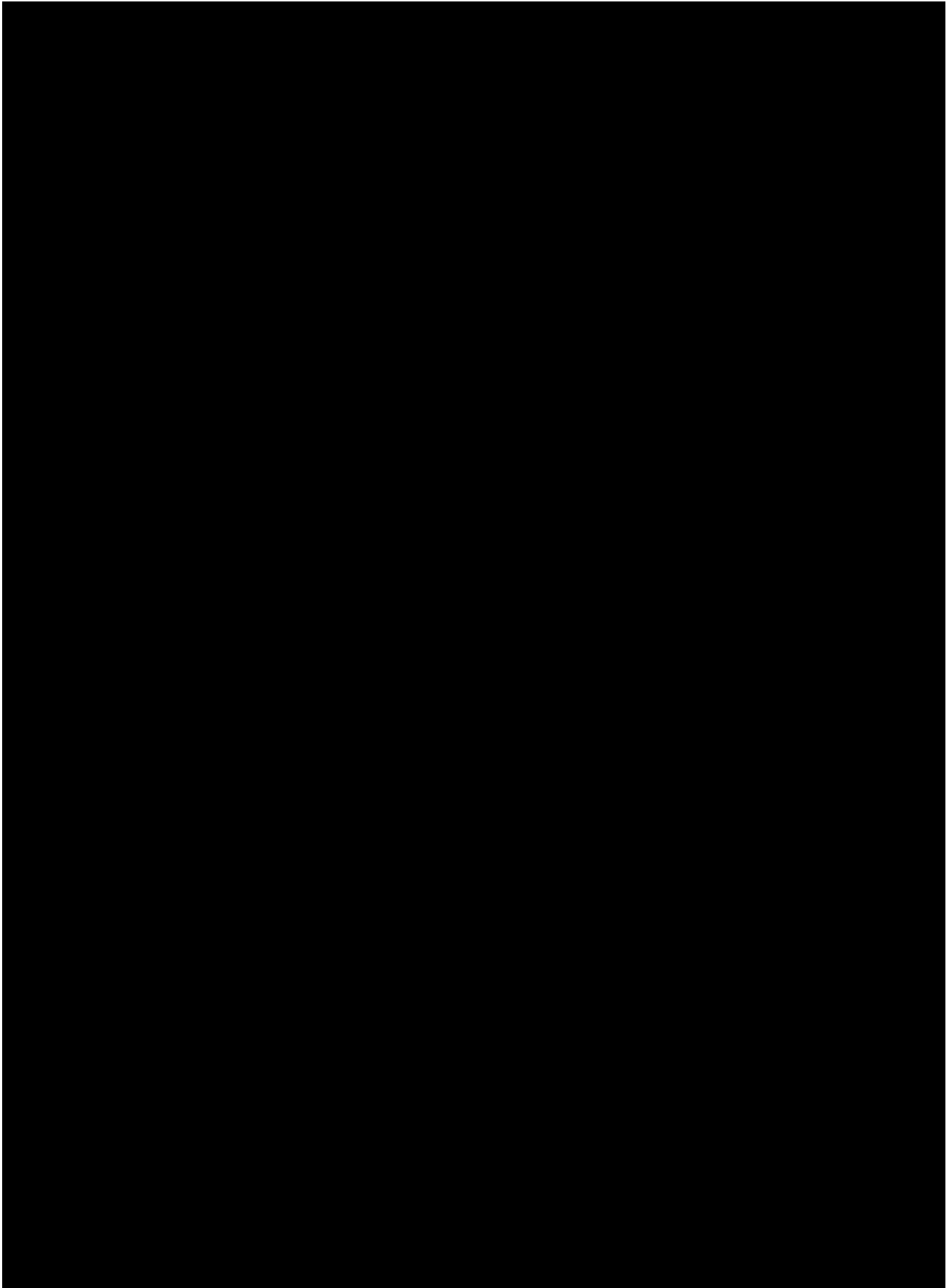


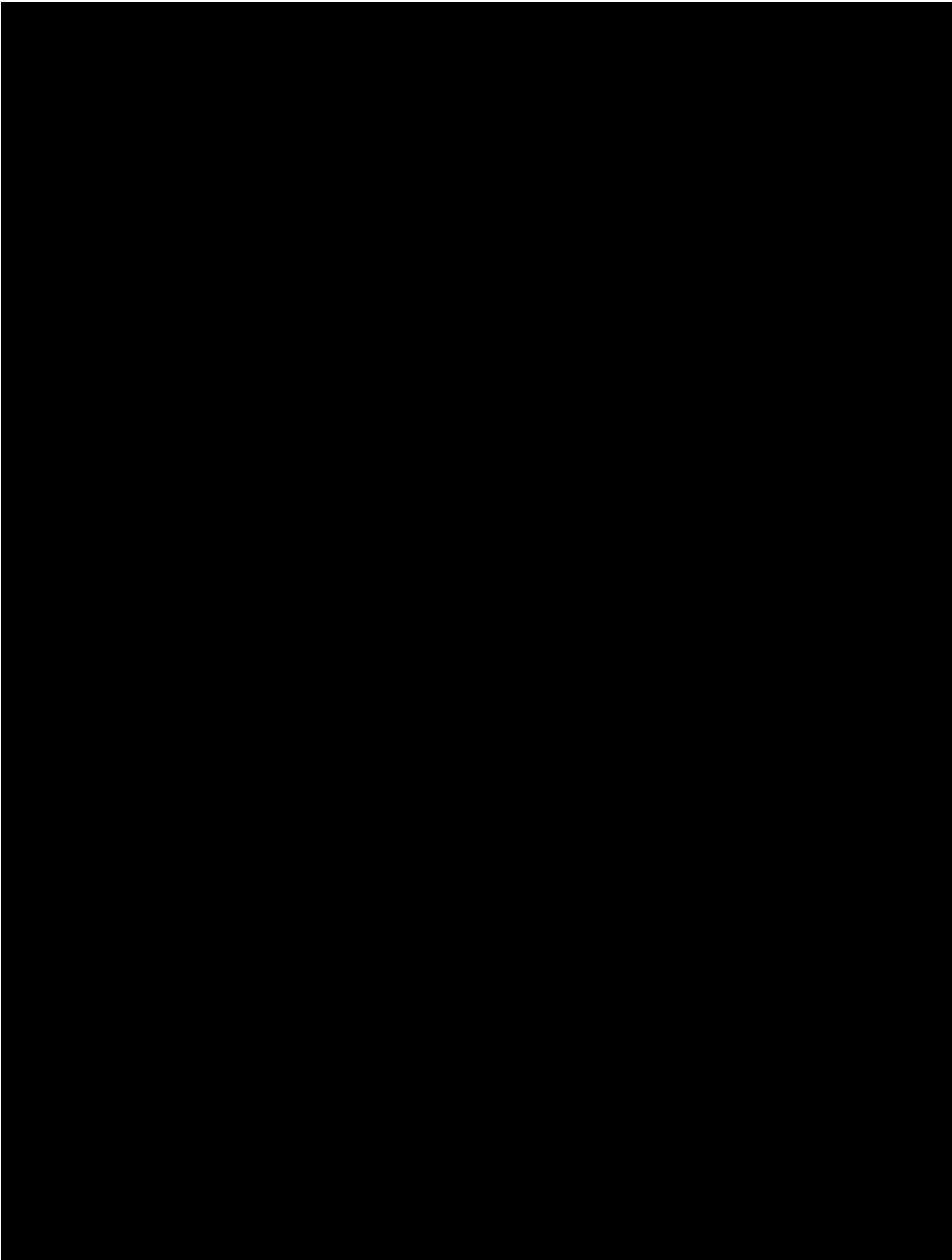


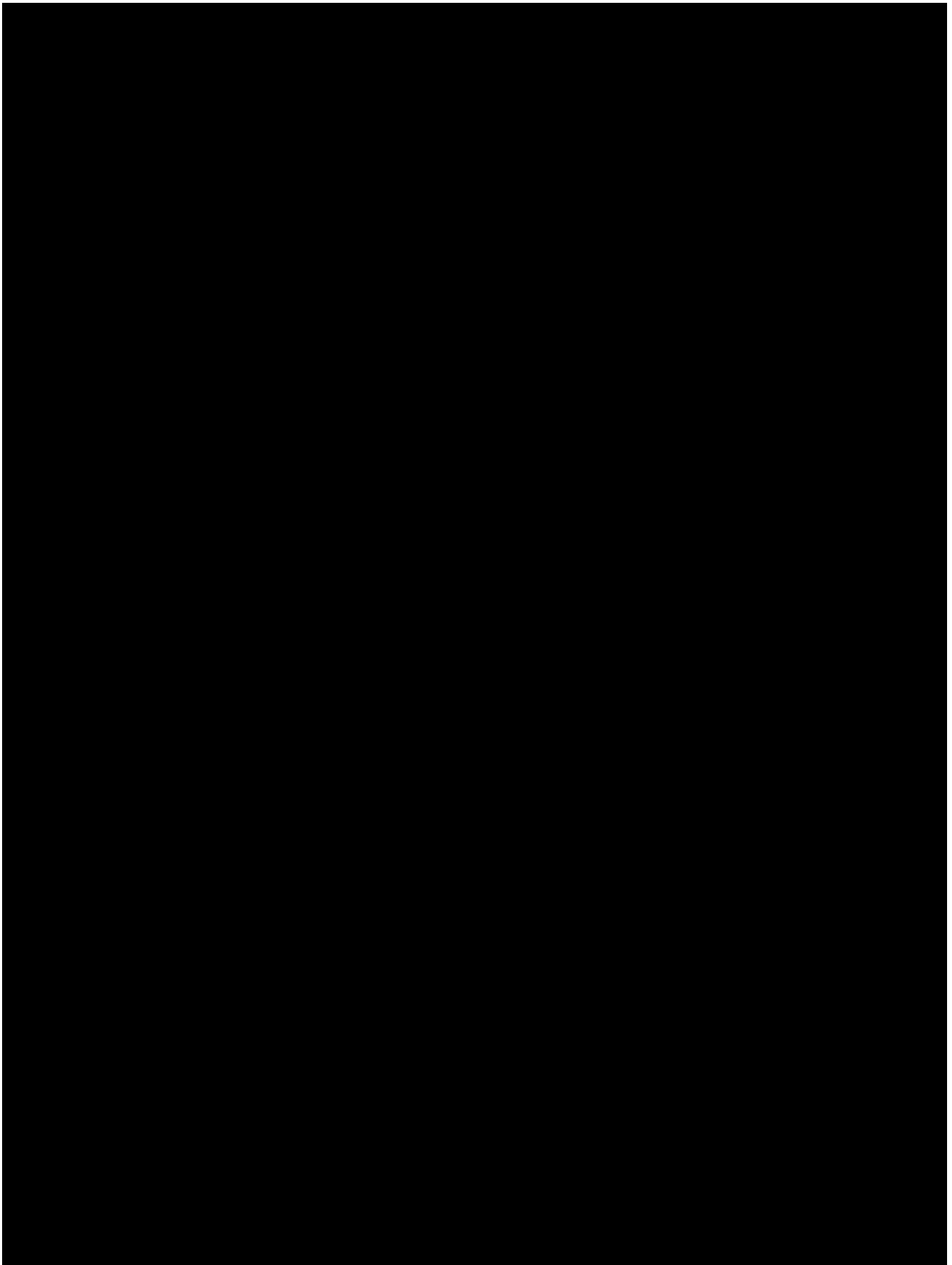


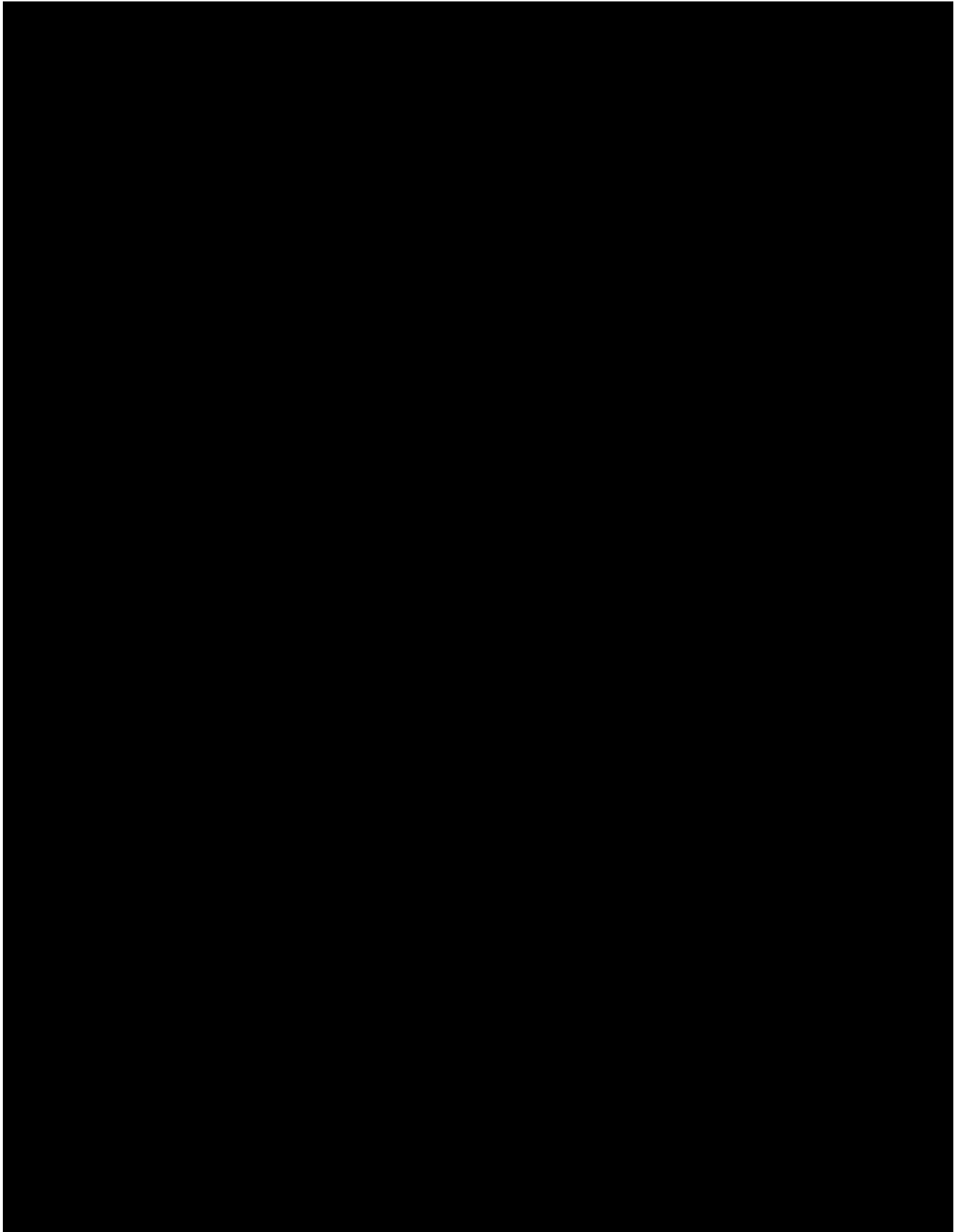


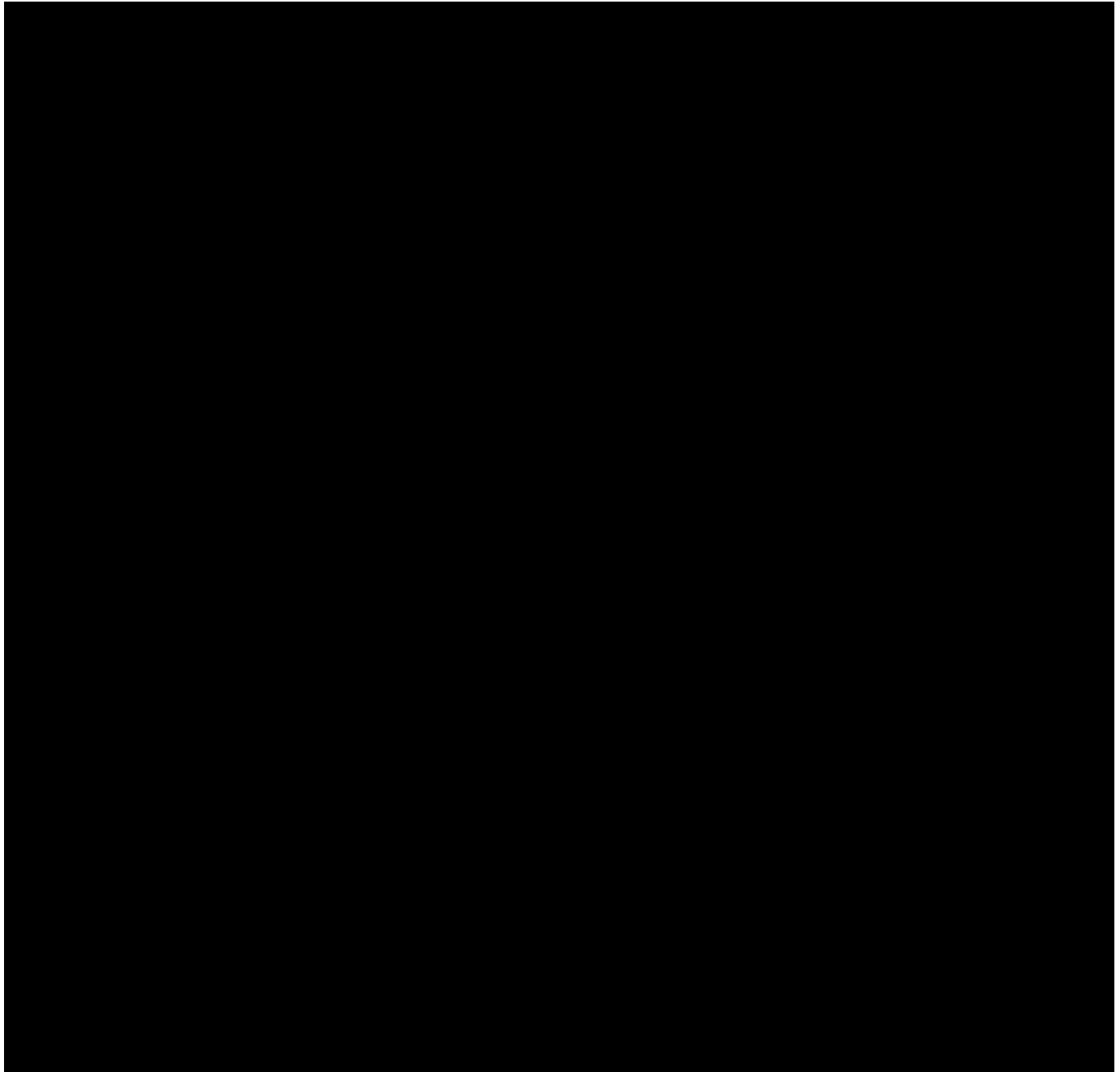













V. CONCLUSION

Defendants' Motion to Amend is **GRANTED IN PART** and **DENIED IN PART** for the reasons stated herein.

So ORDERED and SIGNED this 17th day of October, 2023.



RODNEY GILSTRAP
UNITED STATES DISTRICT JUDGE

**IN THE UNITED STATES DISTRICT COURT
FOR THE EASTERN DISTRICT OF TEXAS
MARSHALL DIVISION**

SEAGEN INC.,

Plaintiff,

V.

DAIICHI SANKYO CO., LTD.,

Defendant,

ASTRAZENECA PHARMACEUTICALS
LP, and ASTRAZENECA UK LTD

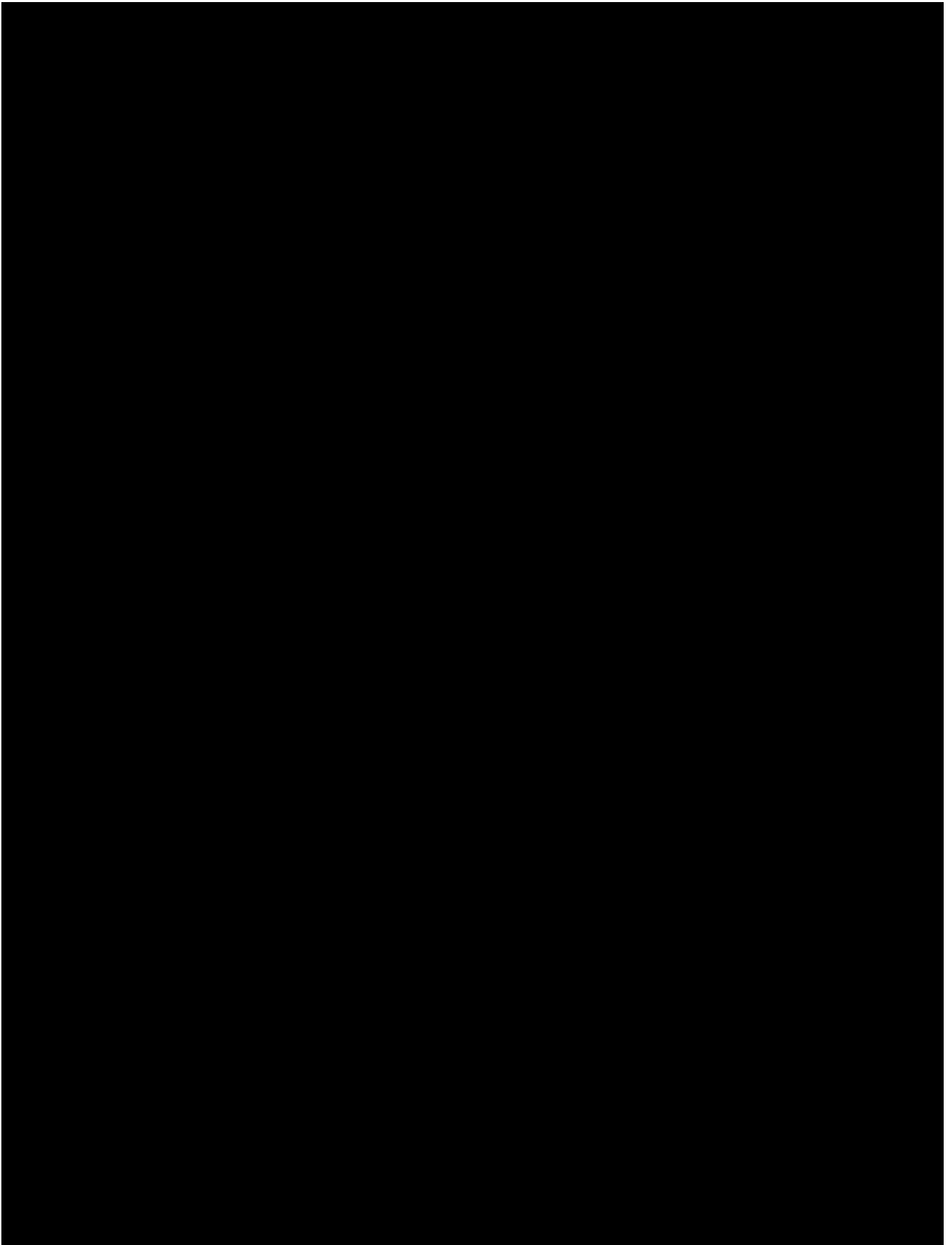
Intervenor-Defendants.

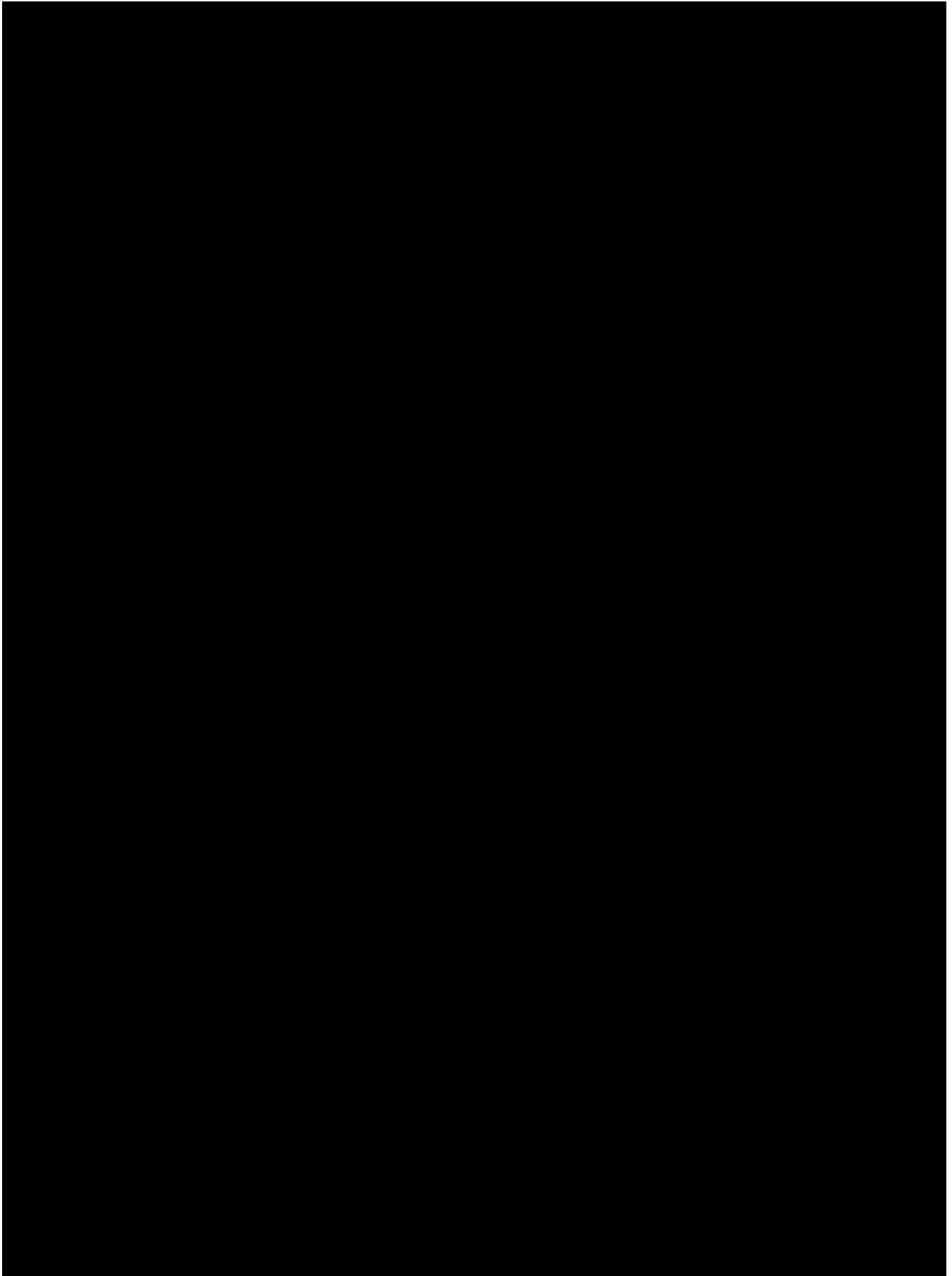
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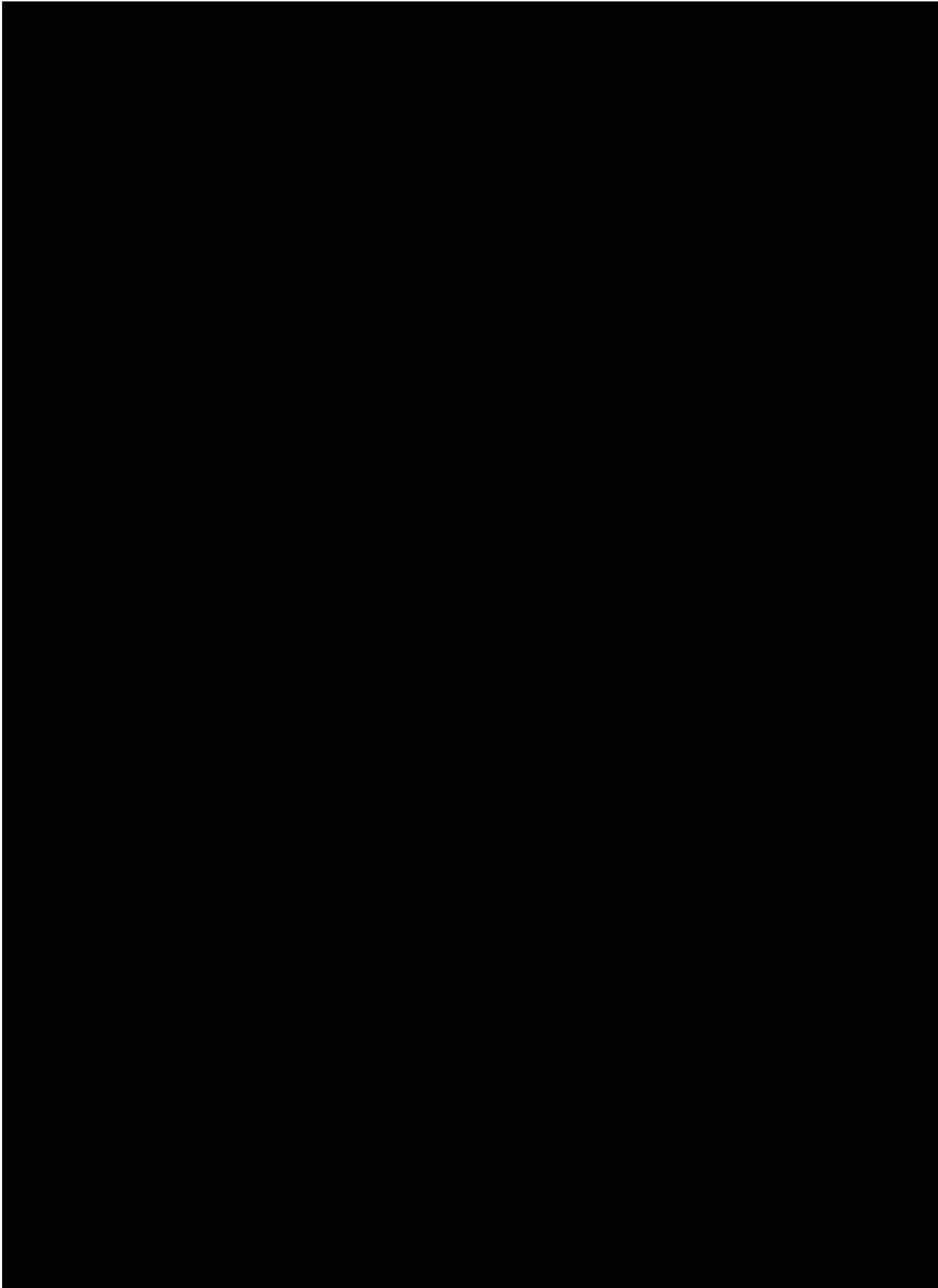
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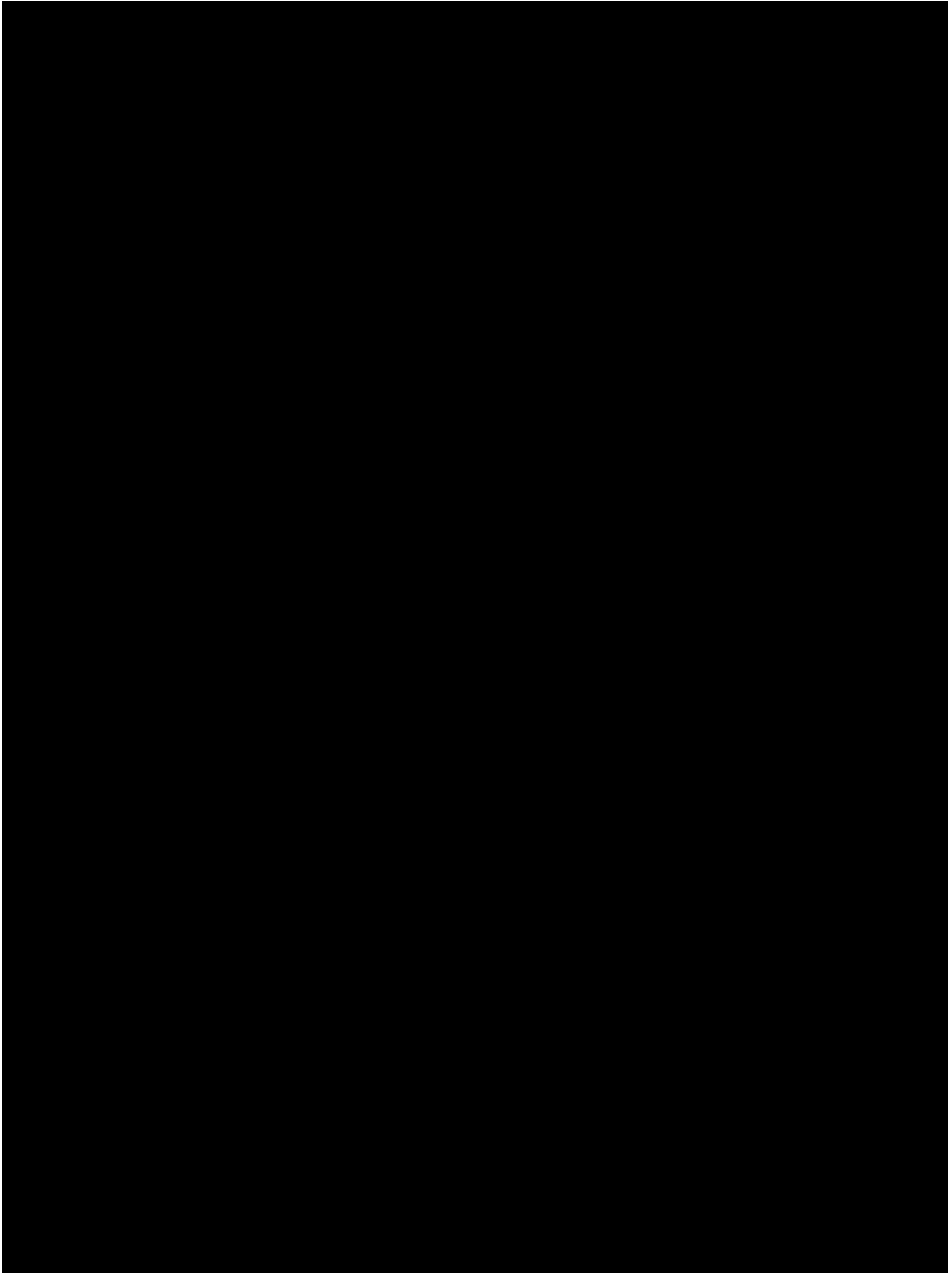
MEMORANDUM OPINION AND ORDER

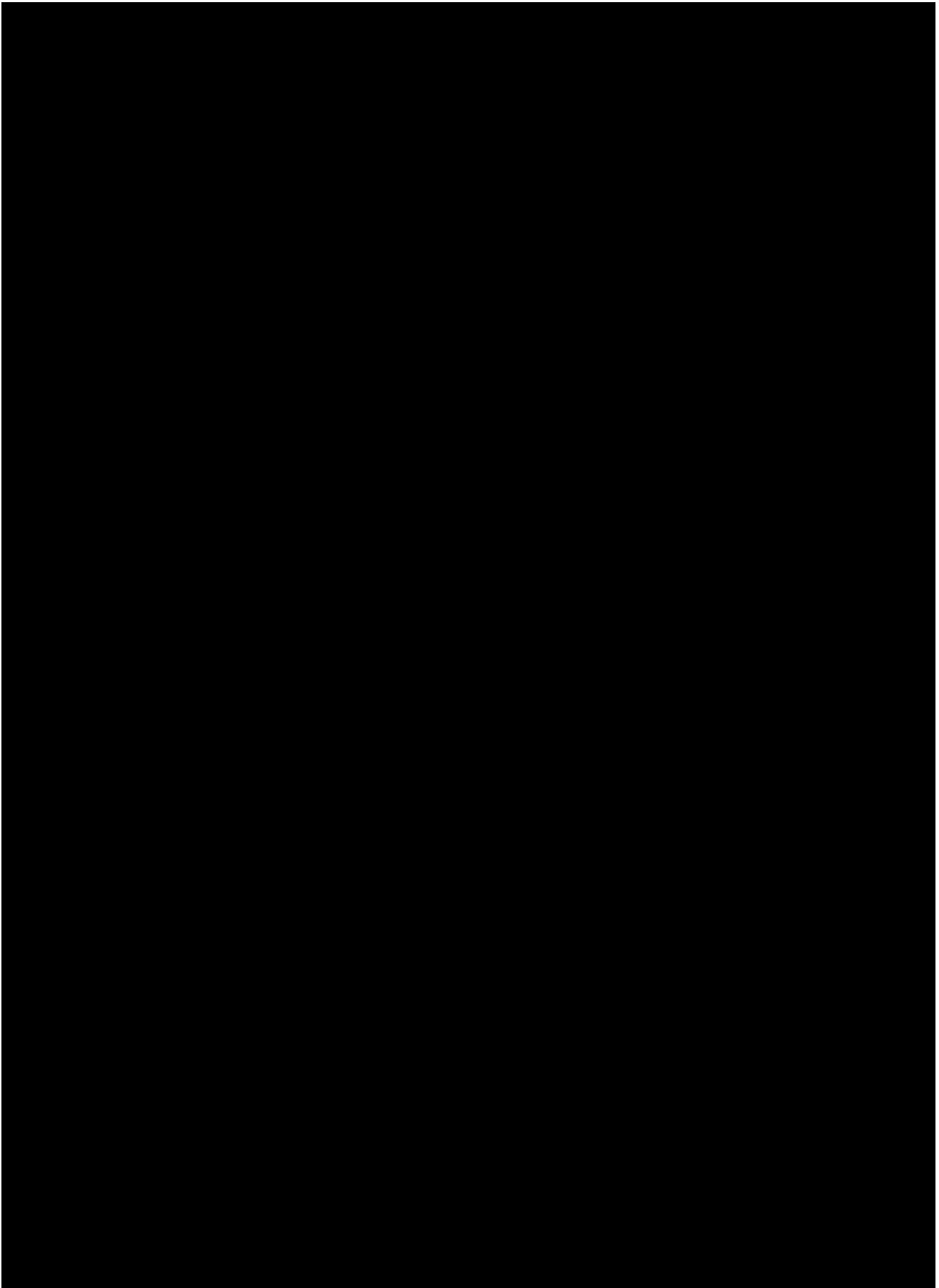
Before the Court are Defendants Daiichi Sankyo Co., Ltd. (“DSC”), AstraZeneca Pharmaceuticals LP, and AstraZeneca UK Ltd. (collectively, “Defendants”) Renewed Motion for Judgment of Invalidity as a Matter of Law (the “Invalidity JMOL Motion”) (Dkt. No. 444) and Renewed Motion for Judgment as a Matter of Law as to Non-Infringement and Damages (the “Infringement/Damages JMOL Motion”) (Dkt. No. 445) (collectively, “Motions”). Having considered the Motions, the Court is of the opinion that they should be **DENIED**.

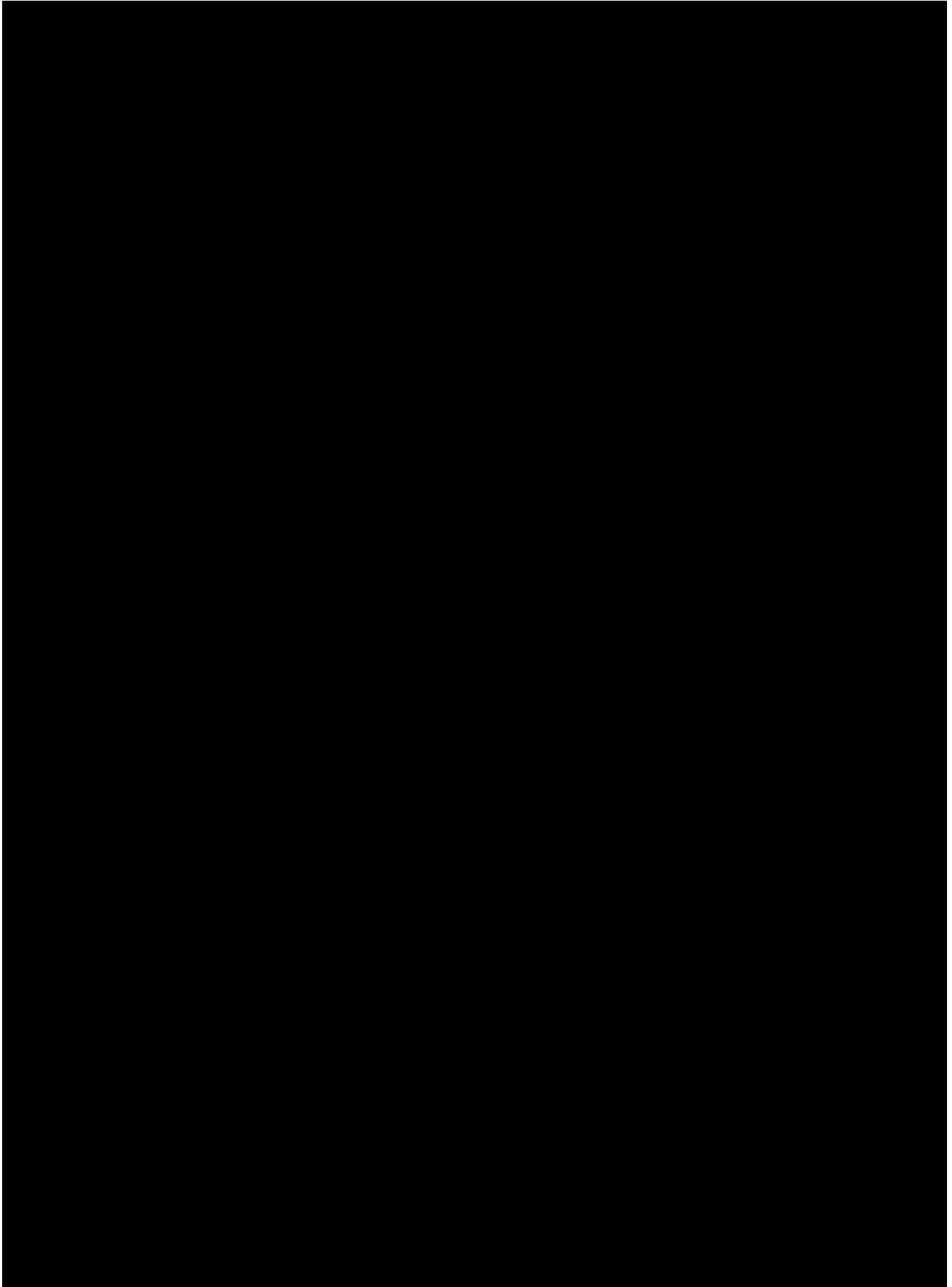


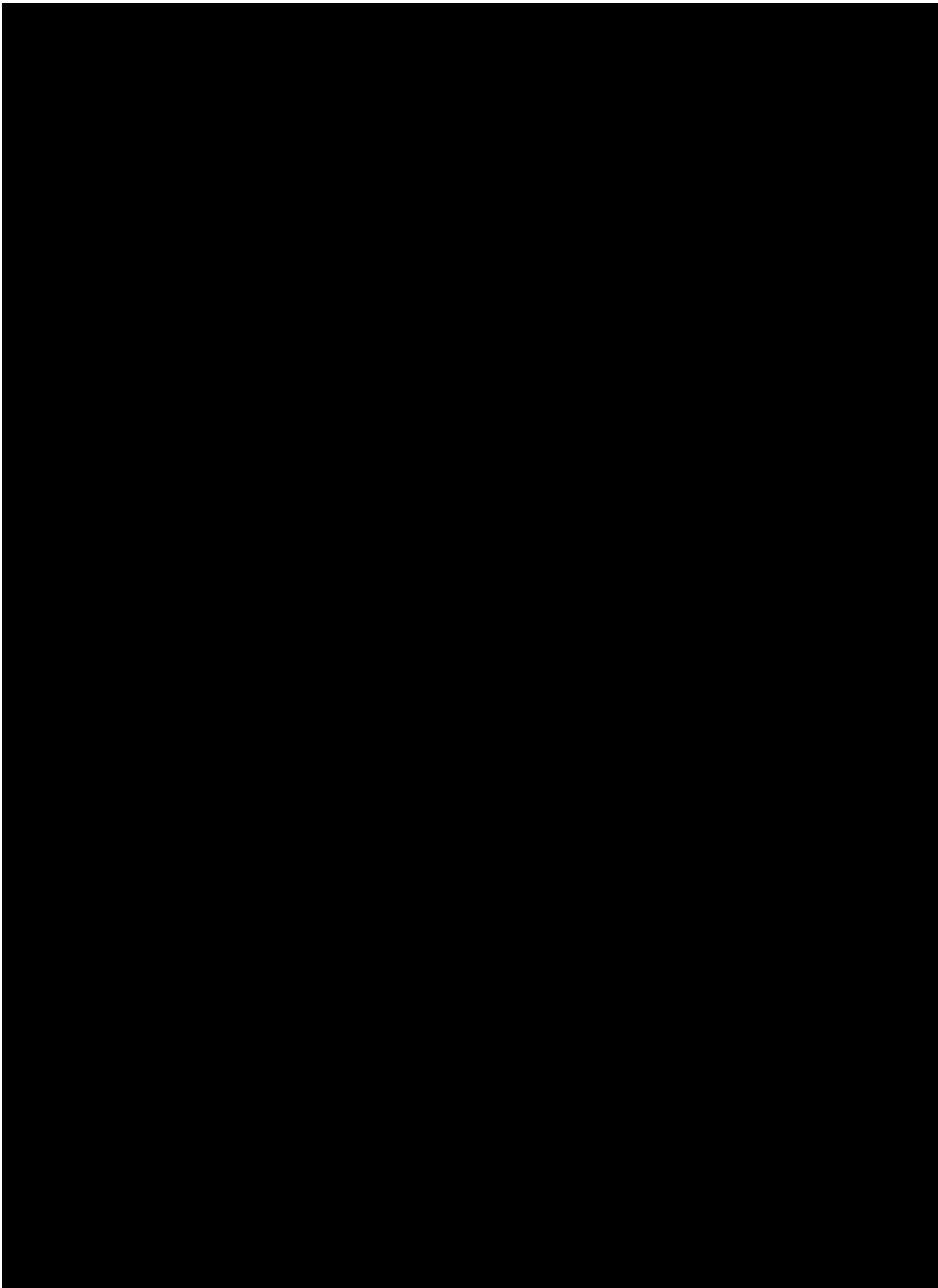


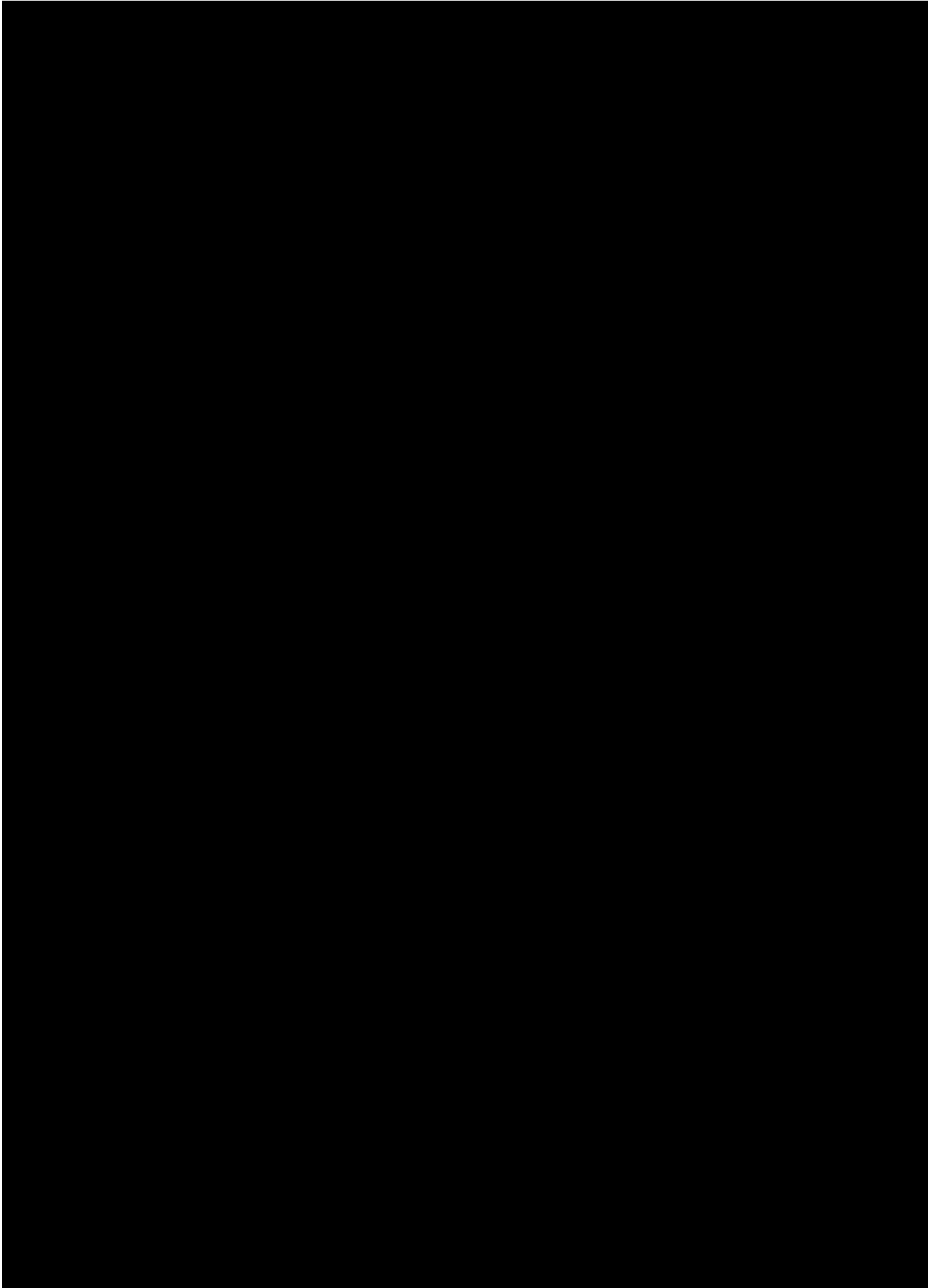


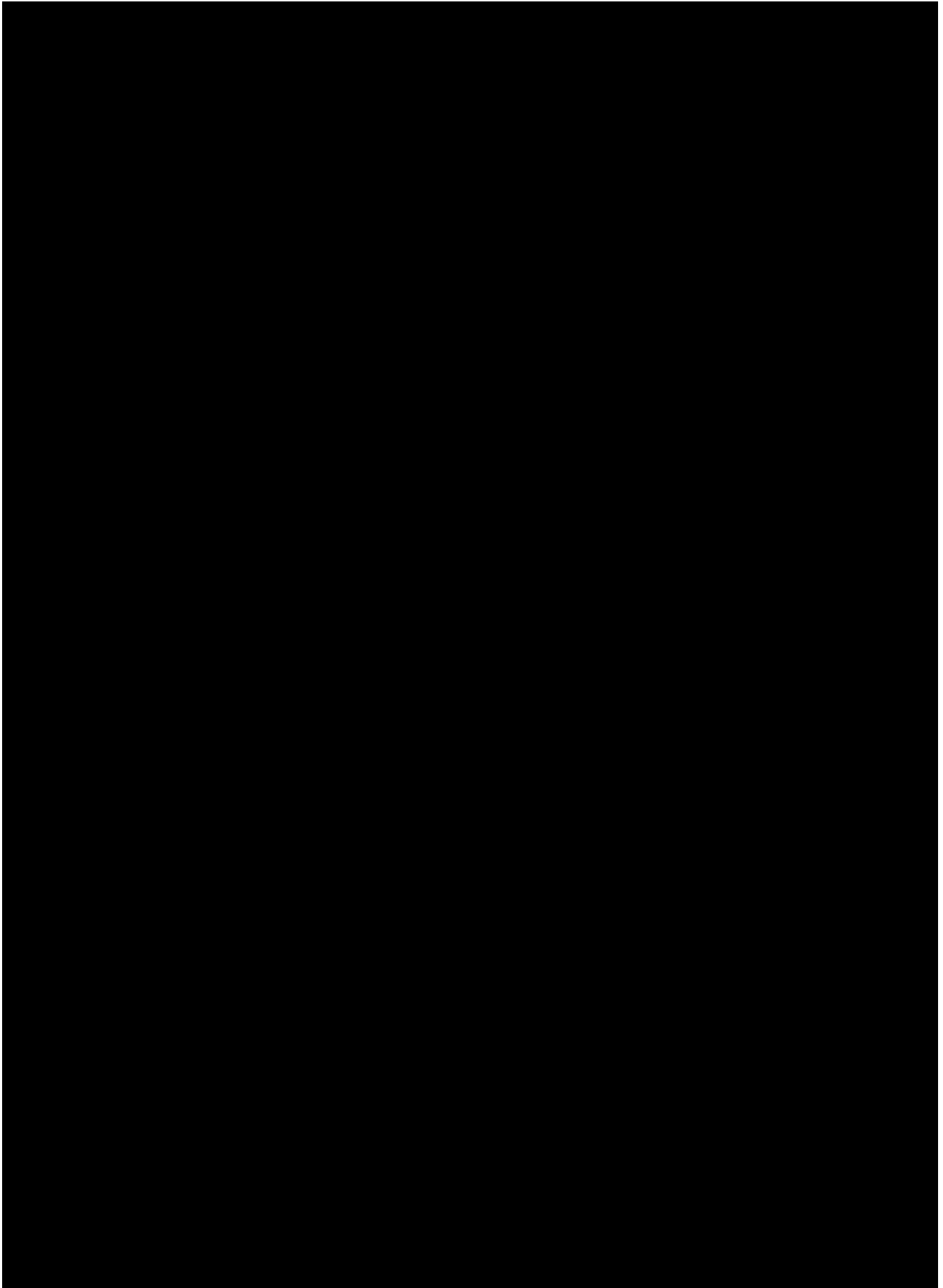


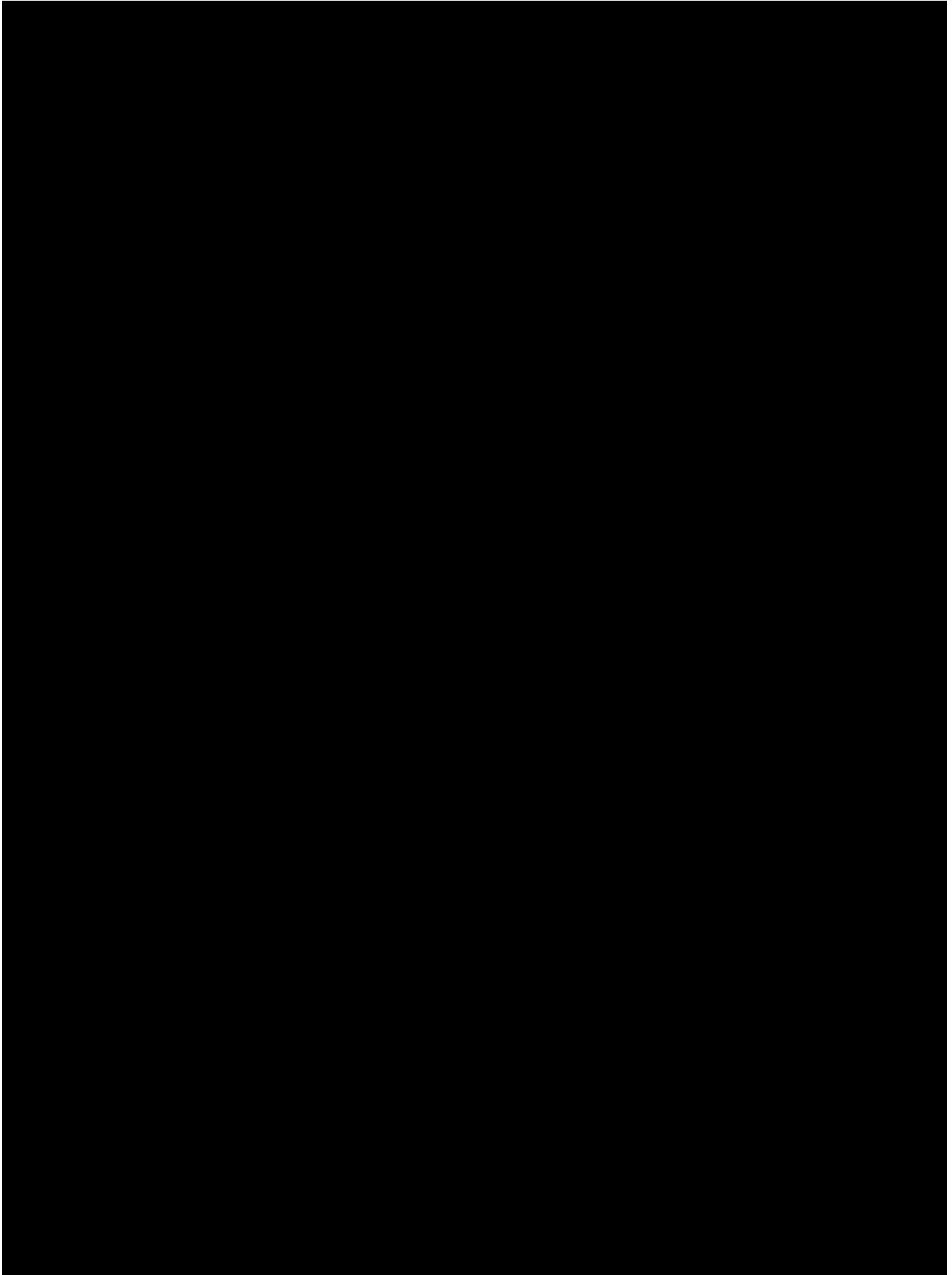


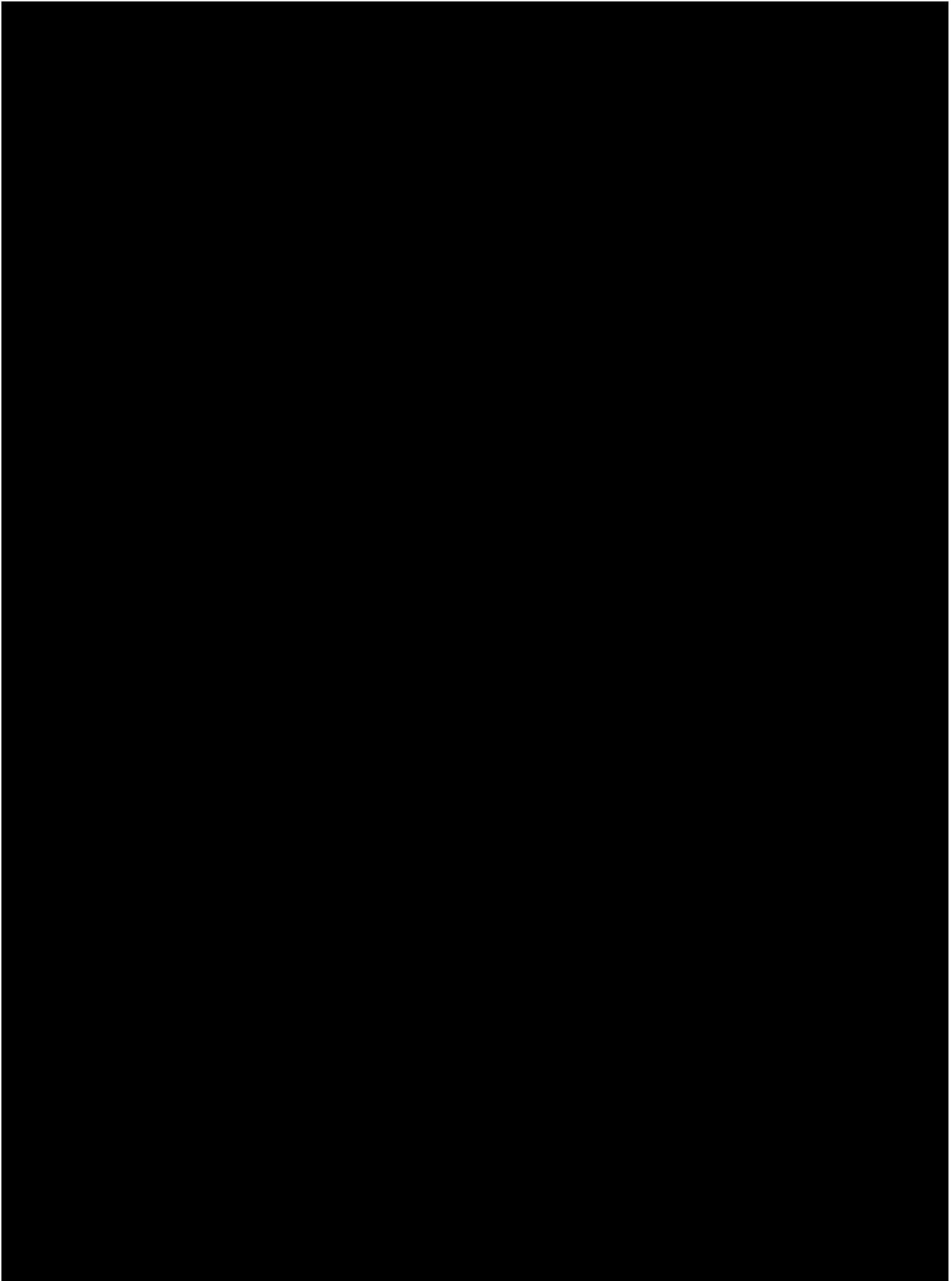


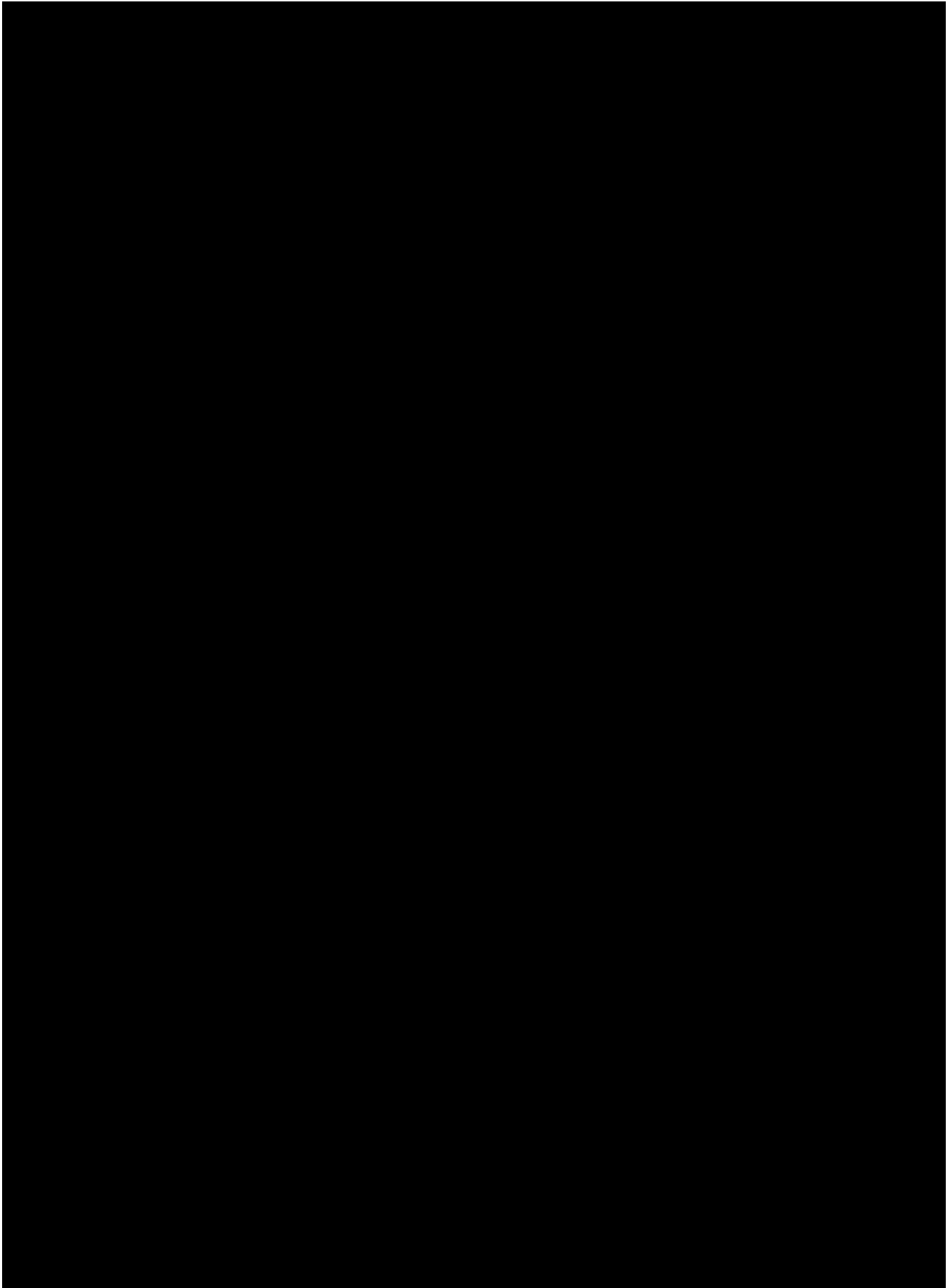


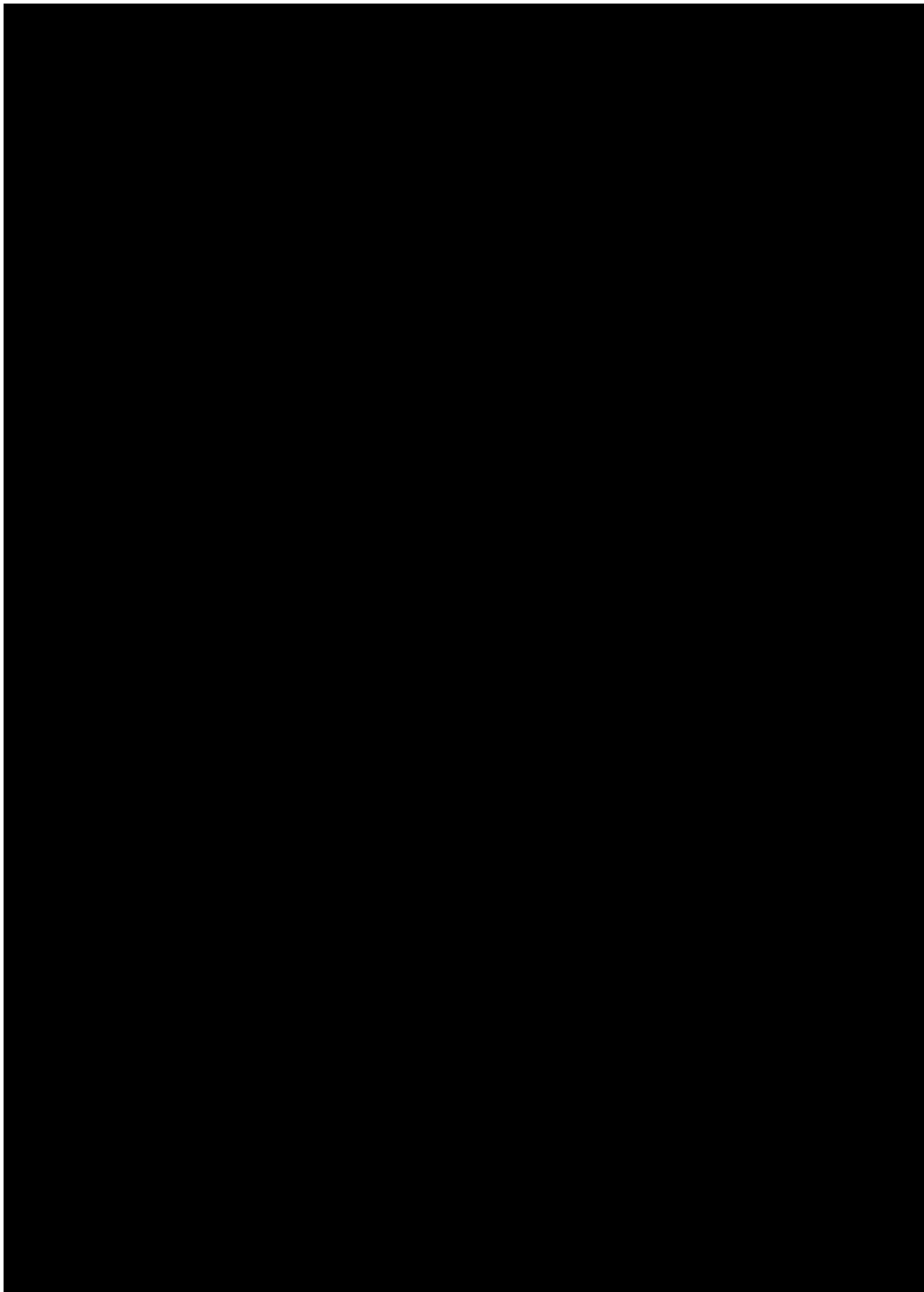


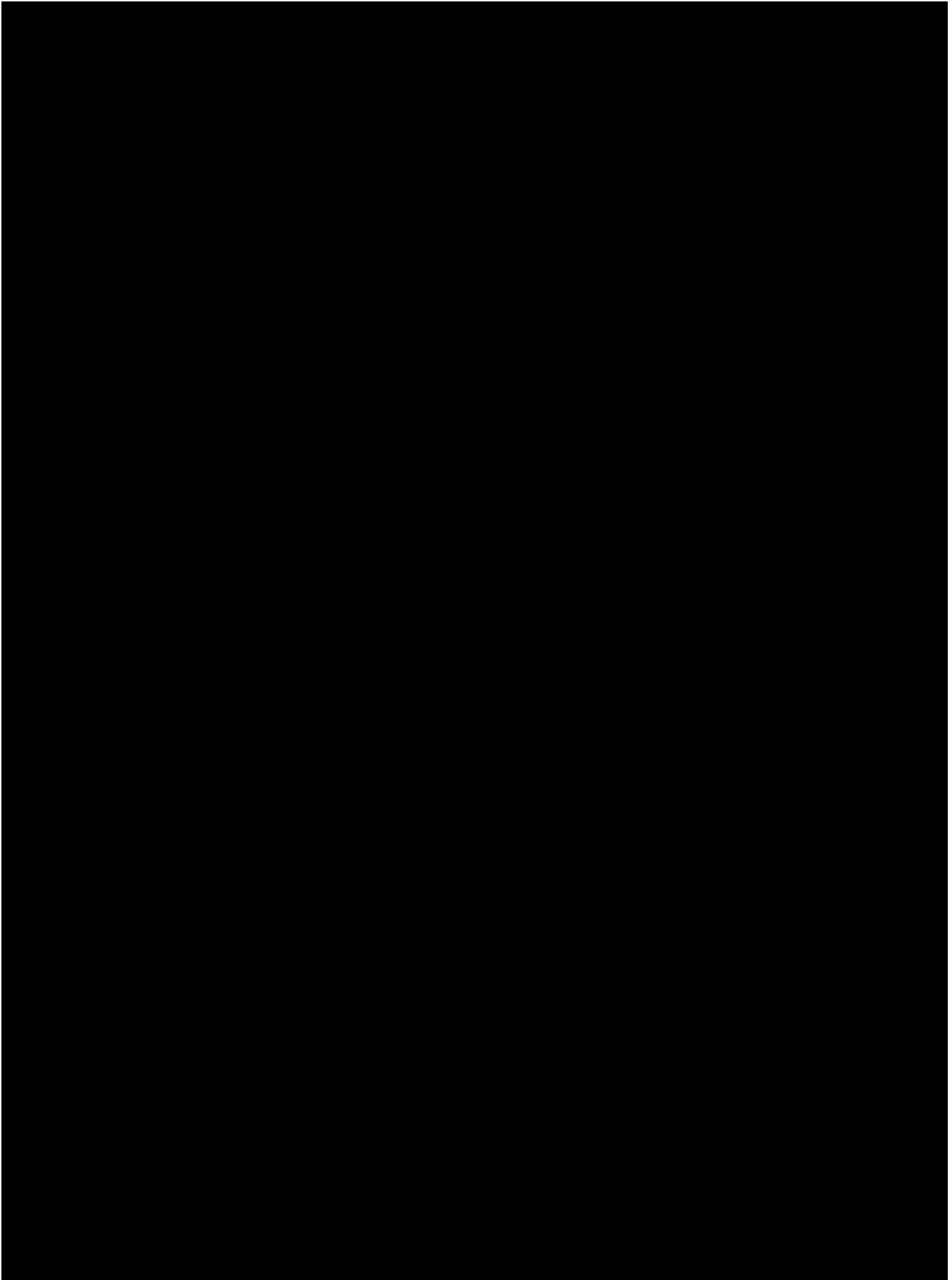


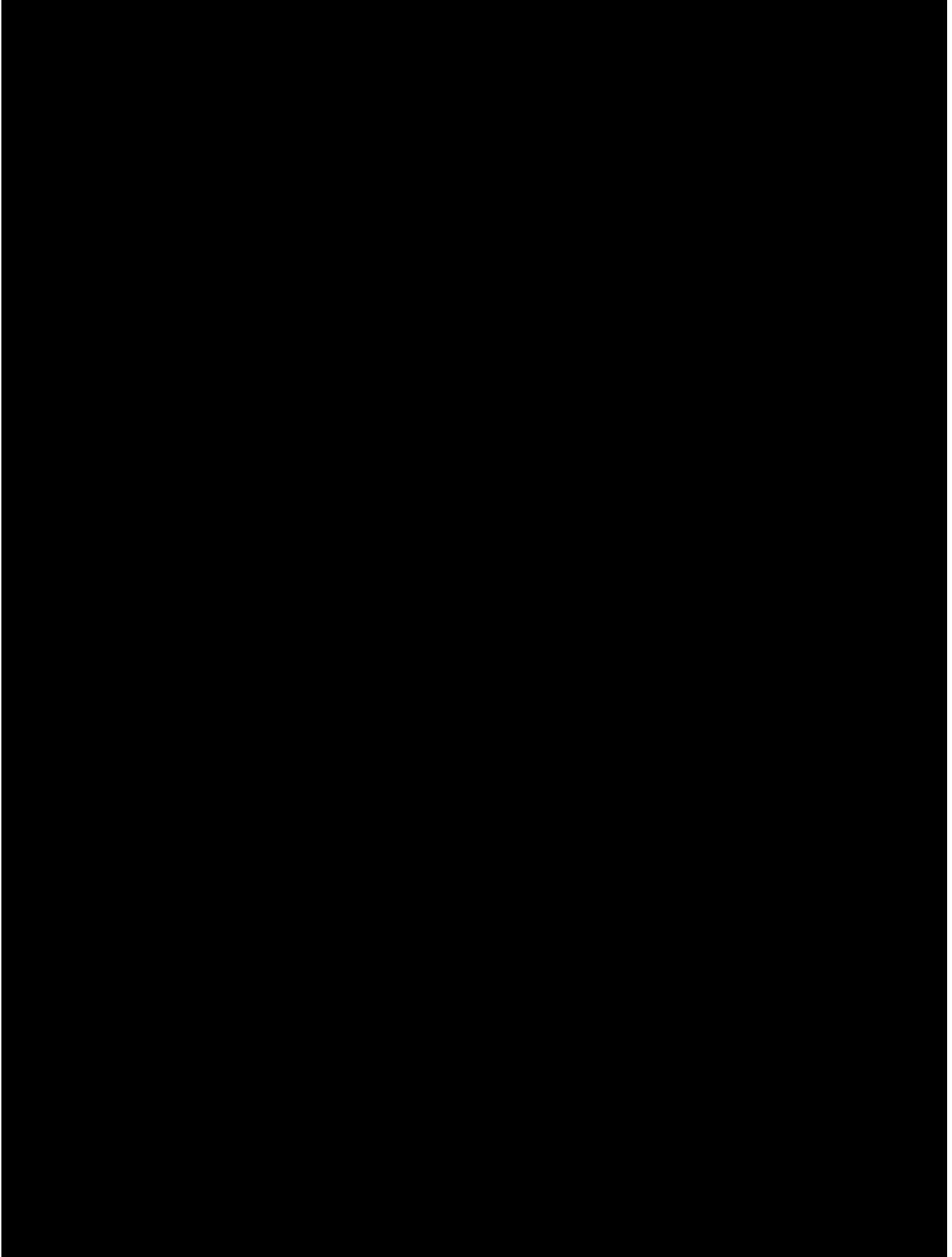


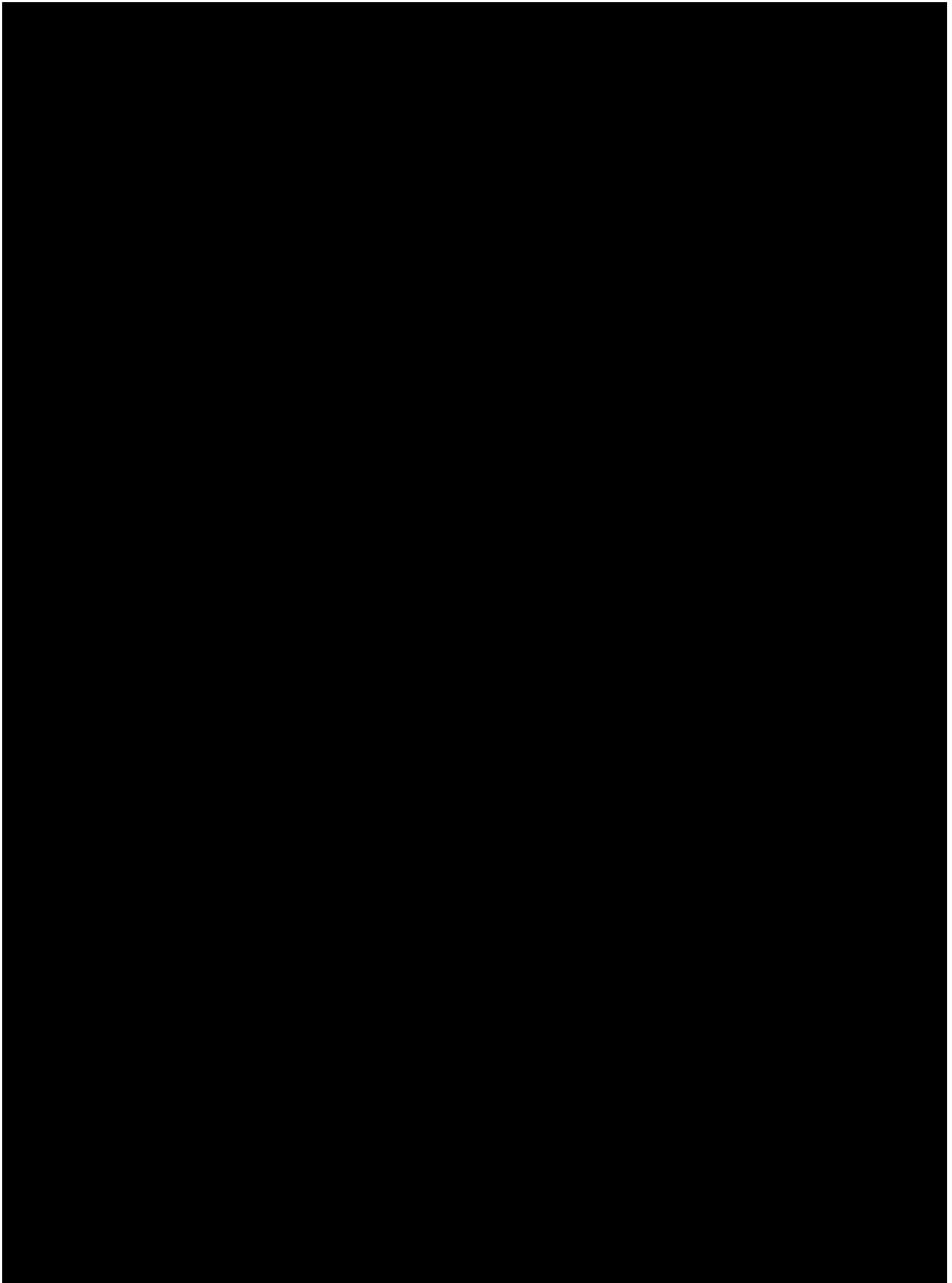


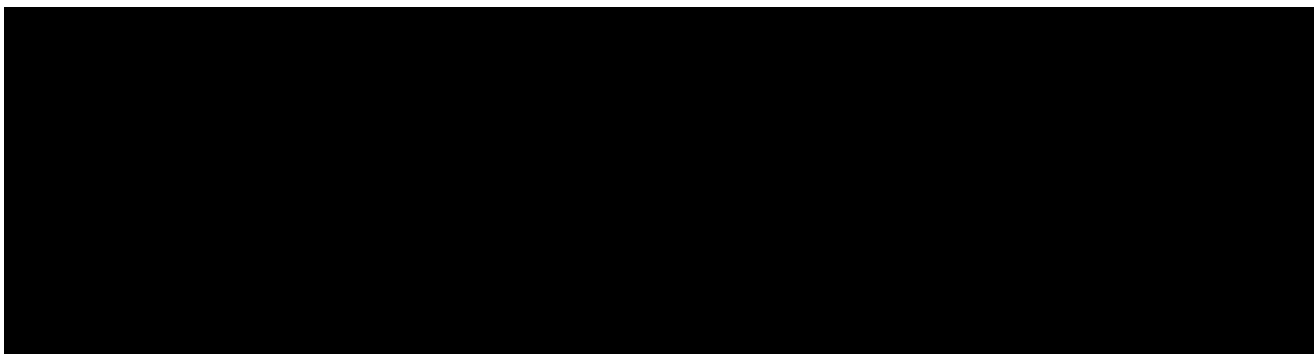








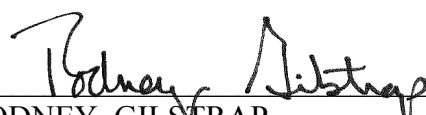




IV. CONCLUSION

For the reasons set forth herein, Defendants' Renewed Motion for Judgment of Invalidity as a Matter of Law (Dkt. No. 444) and Renewed Motion for Judgment as a Matter of Law as to Non-Infringement and Damages (Dkt. No. 445) are **DENIED**.

So ORDERED and SIGNED this 21st day of August, 2023.



RODNEY GILSTRAP
UNITED STATES DISTRICT JUDGE

Pursuant to Rule 58 of the Federal Rules of Civil Procedure, and in accordance with the jury's unanimous verdict and the entirety of the record, the Court hereby **ORDERS** and **ENTERS JUDGMENT** as follows:

1. DSC has infringed at least one of the Asserted Claims;
2. The Asserted Claims are not invalid;
3. DSC's infringement was willful;
4. Seagen is hereby awarded damages from and against DSC and shall accordingly have and recover from DSC the sum of \$41,820,000.00 U.S. Dollars as a reasonable royalty for sales from October 20, 2020 through March 31, 2022;
5. Notwithstanding the jury's finding of willfulness, the Court having considered the totality of the circumstances together with the material benefit of having presided throughout the jury trial and having seen the same evidence and heard the same arguments as the jury, and mindful that enhancement is generally reserved for "egregious cases of culpable behavior,"¹ concludes that enhancement of the compensatory award herein is not warranted under 35 U.S.C. § 284 and consequently, the Court elects not to enhance the damages awarded herein;
6. Pursuant to 35 U.S.C. § 284 and Supreme Court guidance that "prejudgment interest shall ordinarily be awarded absent some justification for withholding such an award,"² the Court awards pre-judgment interest applicable to all sums awarded herein, calculated at the 5-year U.S. Treasury Bill rate, compounded quarterly, from the date of infringement through the date of entry of this Judgment;³ and

¹ *Halo Electronics, Inc. v. Pulse Electronics, Inc.*, 136 S.Ct. 1923, 1934 (2016).


² *General Motors Corp. v. Devex Corp.*, 461 U.S. 648, 657 (1983).

³ *See Nickson Indus., Inc. v. Rol Mfg. Co., Ltd.*, 847 F.2d 795, 800–801 (Fed. Cir. 1988).

7. Pursuant to 28 U.S.C. § 1961, the Court awards post-judgment interest applicable to all sums awarded herein, at the statutory rate, from the date of entry of this Judgment until paid.
8. Pursuant to Federal Rule of Civil Procedure 54(d), Local Rule CV-54, and 28 U.S.C. § 1920, Seagen is the prevailing party in this case and shall recover its costs from DSC. Seagen is directed to file its proposed Bill of Costs.

All other requests for relief now pending and requested by either party but not specifically addressed herein are **DENIED**.

So ORDERED and SIGNED this 19th day of July, 2022.



RODNEY GILSTRAP
UNITED STATES DISTRICT JUDGE

**IN THE UNITED STATES DISTRICT COURT
FOR THE EASTERN DISTRICT OF TEXAS
MARSHALL DIVISION**

SEAGEN INC.,

Plaintiff,

V.

DAIICHI SANKYO CO., LTD.,

Defendant,

ASTRAZENECA PHARMACEUTICALS
LP, and ASTRAZENECA UK LTD

Intervenor-Defendants.

[illegible]

CIVIL ACTION NO. 2:20-CV-00337-JRG

VERDICT FORM

In answering the following questions and completing this Verdict Form, you are to follow all the instructions that I have given you in the Court's Final Jury Instructions. Your answers to each question must be unanimous. Some of the questions contain legal terms that are defined and explained in detail in the Final Jury Instructions. You should refer to and consider the Final Jury Instructions as you answer the questions in this Verdict Form.

As used herein, the following terms have the following meanings:

- “**Plaintiff**” or “**Seagen**” refers to Seagen Inc.
- “**Daiichi Sankyo**” or “**DSC**” refers to Daiichi Sankyo Company, Limited.
- “**AstraZeneca**” or “**AZ**” refers collectively to AstraZeneca Pharmaceutical LP and AstraZeneca UK Ltd.
- “**Defendants**” refers collectively to DSC and AZ
- The “**’039 Patent**” refers to U.S. Patent No. 10,808,039.
- The “**Asserted Claims**” refers collectively to Claims 1 through 5, 9, and 10 of the ’039 Patent.

**IT IS VERY IMPORTANT THAT YOU FOLLOW THE
INSTRUCTIONS PROVIDED IN THIS VERDICT FORM**

**READ THEM CAREFULLY AND ENSURE THAT YOUR
VERDICT COMPLIES WITH THEM**

QUESTION NO. 1

Did Seagen, the Plaintiff, prove by a preponderance of the evidence that Defendant DSC, infringed **ANY** of the Asserted Claims?

Yes: ✓ No: _____

QUESTION NO. 2

Did DSC and AZ, the Defendants, prove by clear and convincing evidence that any of the following Asserted Claims are invalid?

For each claim below, please answer "Yes" or "No".

Claim 1 of the '039 Patent:	Yes: _____	No: <u>✓</u>
Claim 2 of the '039 Patent:	Yes: _____	No: <u>✓</u>
Claim 3 of the '039 Patent:	Yes: _____	No: <u>✓</u>
Claim 4 of the '039 Patent:	Yes: _____	No: <u>✓</u>
Claim 5 of the '039 Patent:	Yes: _____	No: <u>✓</u>
Claim 9 of the '039 Patent:	Yes: _____	No: <u>✓</u>
Claim 10 of the '039 Patent:	Yes: _____	No: <u>✓</u>

If you answered “NO” to Question No. 1, OR “YES” to ALL Asserted Claims in Question No. 2, then DO NOT answer Question No. 3.

Answer Question No. 3 ONLY as to any Asserted Claim that you have found BOTH to be infringed AND not invalid.

QUESTION NO. 3

Did Seagen, the Plaintiff, prove by a preponderance of the evidence that Defendant DSC willfully infringed ANY of the Asserted Claims that you found were infringed?

Yes: ✓ No: _____

If you answered “NO” to Question No. 1, OR “YES” to ALL Asserted Claims in Question No. 2, then DO NOT answer Question No. 4.

Answer Question No. 4 ONLY as to any Asserted Claim that you have found BOTH to be infringed AND not invalid.

QUESTION NO. 4

What sum of money, if paid now in cash, has Seagen, the Plaintiff, proven by a preponderance of the evidence would compensate it for its damages for infringement from October 20, 2020 through March 31, 2022?

Answer in United States Dollars and Cents, if any:

\$ 41,820,000.00

FINAL PAGE OF THE JURY VERDICT FORM

You have now reached the end of the Verdict Form and should review it to ensure that it accurately reflects your unanimous determinations. The Jury Foreperson should then sign and date the Verdict Form in the spaces below. Once this is done, notify the Court Security Officer that you have reached a verdict. The Jury Foreperson should keep the Verdict Form and bring it when the jury is brought back into the courtroom.

Signed this 8 day of April 2022.

Jury Foreperson



US010808039B2

(12) United States Patent
Doronina et al.**(10) Patent No.: US 10,808,039 B2****(45) Date of Patent: Oct. 20, 2020****(54) MONOMETHYLVALINE COMPOUNDS**
CAPABLE OF CONJUGATION TO LIGANDS**(71) Applicant: SEATTLE GENETICS, INC.**, Bothell, WA (US)**(72) Inventors: Svetlana O. Doronina**, Snohomish, WA (US); **Peter D. Senter**, Seattle, WA (US); **Brian E. Toki**, Shoreline, WA (US); **Toni Beth Kline**, San Francisco, CA (US)**(73) Assignee: Seattle Genetics Inc.**, Bothell, WA (US)**(*) Notice:** Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.**(21) Appl. No.: 16/507,839****(22) Filed: Jul. 10, 2019****(65) Prior Publication Data**
US 2019/0338045 A1 Nov. 7, 2019**Related U.S. Application Data****(60)** Continuation of application No. 15/811,190, filed on Nov. 13, 2017, now Pat. No. 10,414,826, which is a continuation of application No. 15/188,843, filed on Jun. 21, 2016, now abandoned, which is a continuation of application No. 14/194,106, filed on Feb. 28, 2014, now abandoned, which is a continuation of application No. 13/098,391, filed on Apr. 29, 2011, now Pat. No. 8,703,714, which is a continuation of application No. 11/833,954, filed on Aug. 3, 2007, now Pat. No. 7,994,135, which is a division of application No. 10/983,340, filed on Nov. 5, 2004, now Pat. No. 7,498,298.**(60)** Provisional application No. 60/622,455, filed on Oct. 27, 2004, provisional application No. 60/598,899, filed on Aug. 4, 2004, provisional application No. 60/557,116, filed on Mar. 26, 2004, provisional application No. 60/518,534, filed on Nov. 6, 2003.**(51) Int. Cl.**
C07K 16/32 (2006.01)
C07K 7/02 (2006.01)
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A61K 39/00 (2006.01)**(52) U.S. Cl.**
CPC **C07K 16/32** (2013.01); **A61K 47/6803** (2017.08); **A61K 47/6811** (2017.08); **A61K 47/6849** (2017.08); **A61K 47/6851** (2017.08); **A61K 47/6855** (2017.08); **C07K 7/02** (2013.01); **A61K 38/00** (2013.01); **A61K 2039/505** (2013.01); **C07K 2317/24** (2013.01); **Y02A 50/414** (2018.01); **Y02A 50/423** (2018.01); **Y02A 50/469** (2018.01); **Y10T 428/13** (2015.01)**(58) Field of Classification Search**

None

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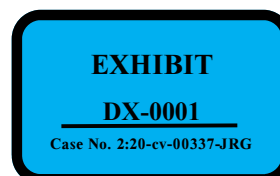
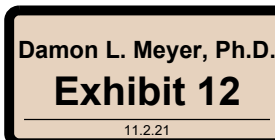
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(74) Attorney, Agent, or Firm — Fish & Richardson P.C.; Min Lin; Paul Naik**(57) ABSTRACT**

Auristatin peptides, including MeVal-Val-Dil-Dap-Norephedrine (MMAE) and MeVal-Val-Dil-Dap-Phe (MMAF), were prepared and attached to Ligands through various linkers, including maleimidocaproyl-val-cit-PAB. The resulting ligand drug conjugates were active in vitro and in vivo.

10 Claims, 40 Drawing Sheets**Specification includes a Sequence Listing.**

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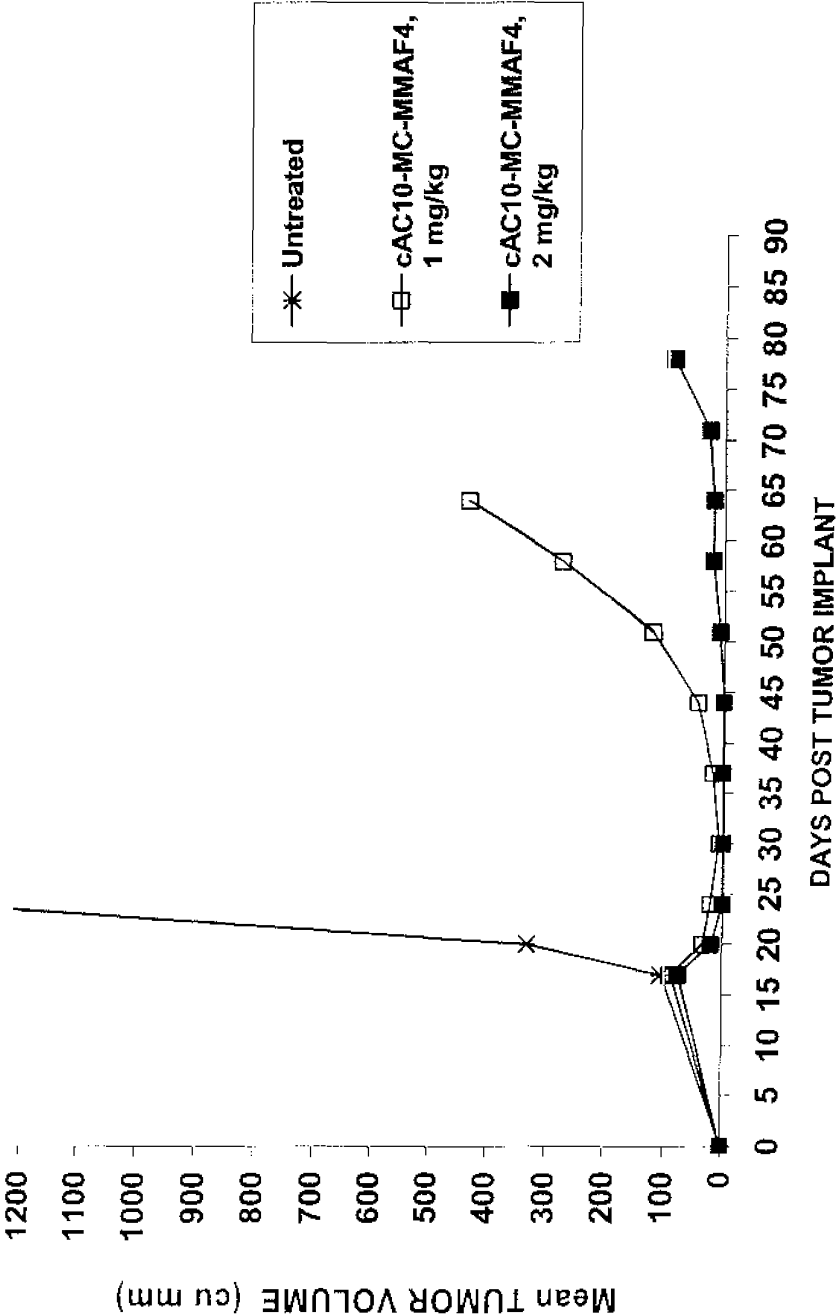


Fig. 1

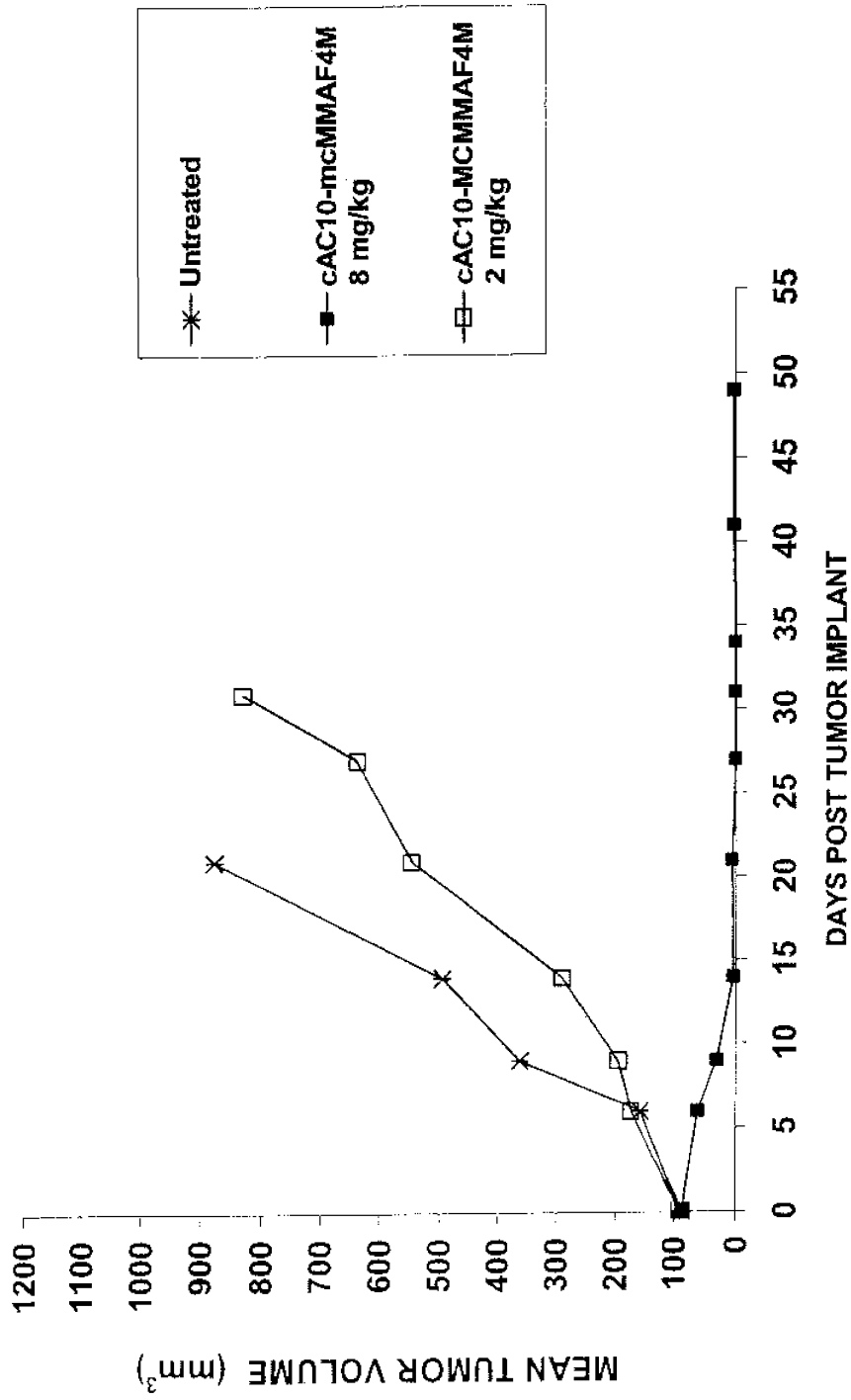


Fig. 2

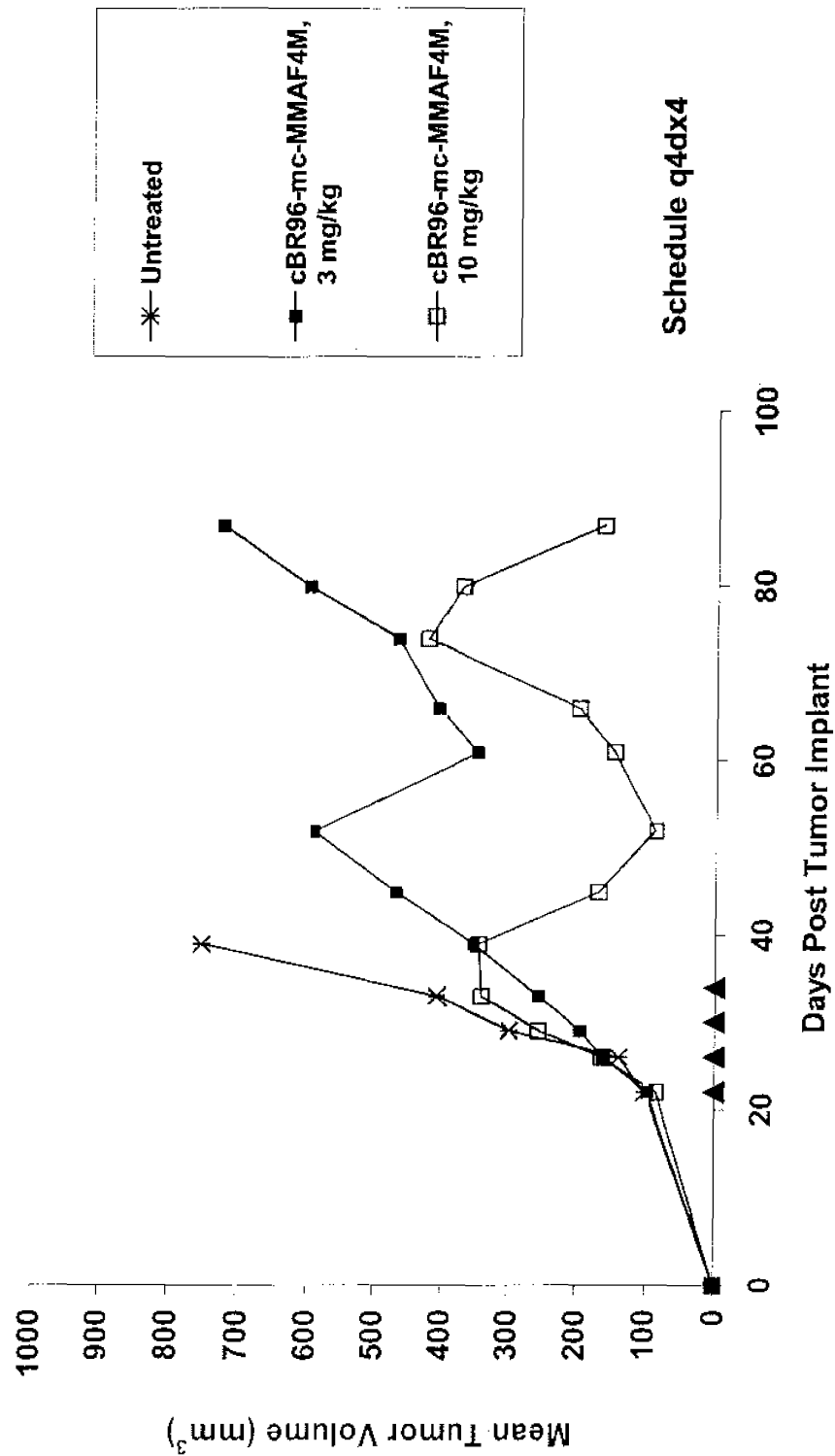


Fig. 3a

Efficacy of mAb-mc-MMAF in L2987 Lung Carcinoma

L2987-AO1

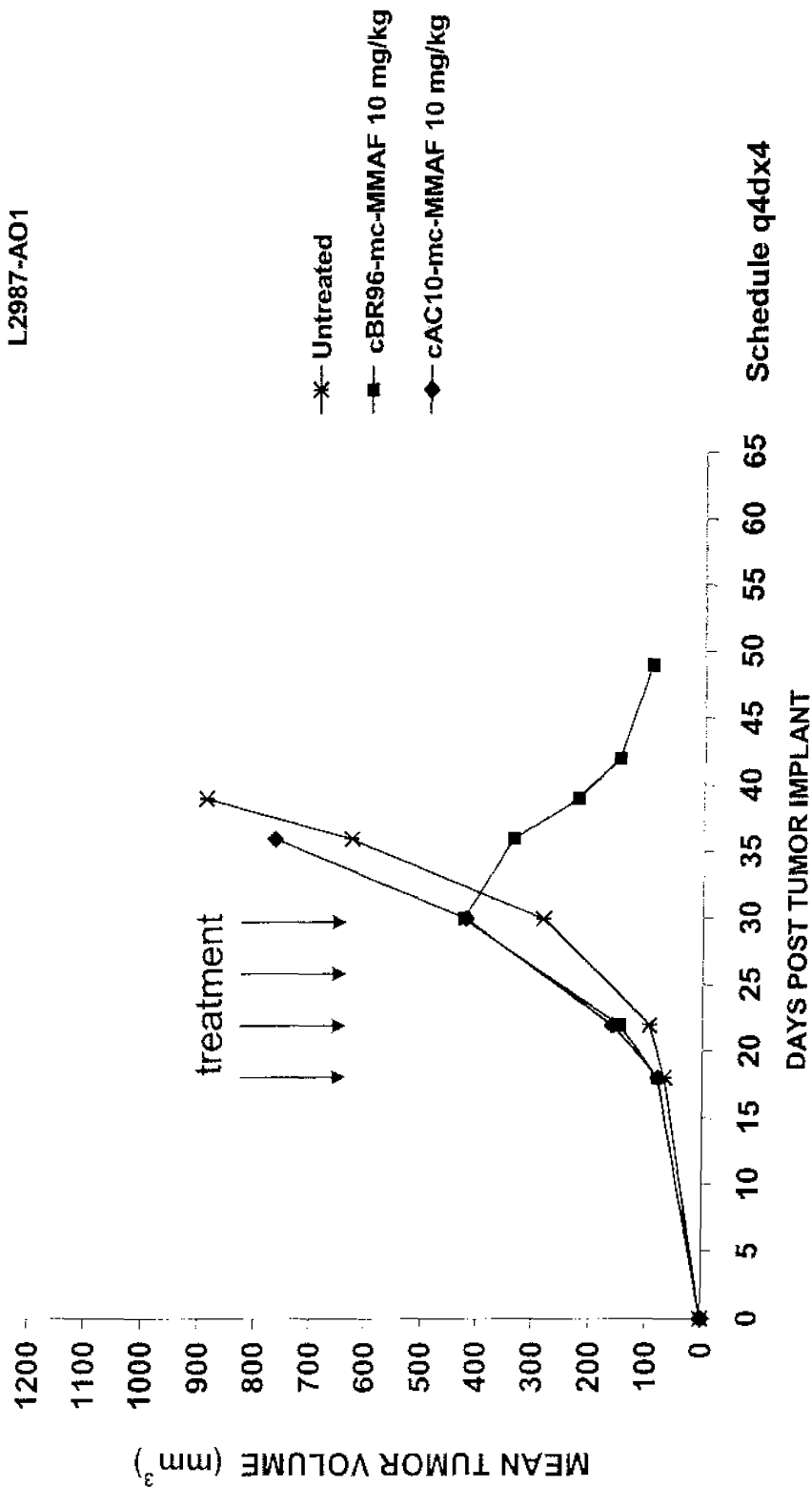


Fig. 3b

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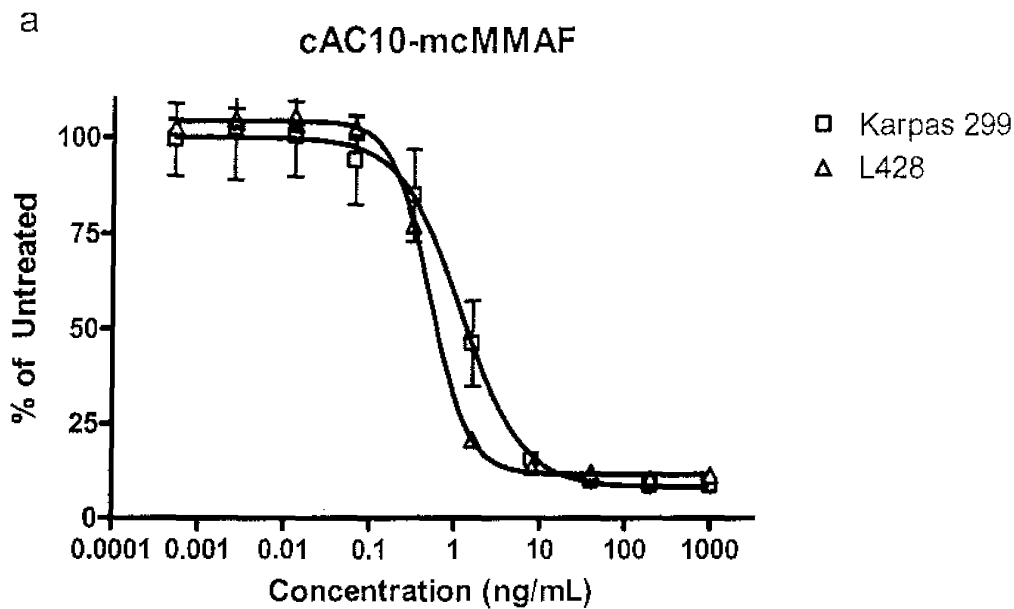


Fig. 4a

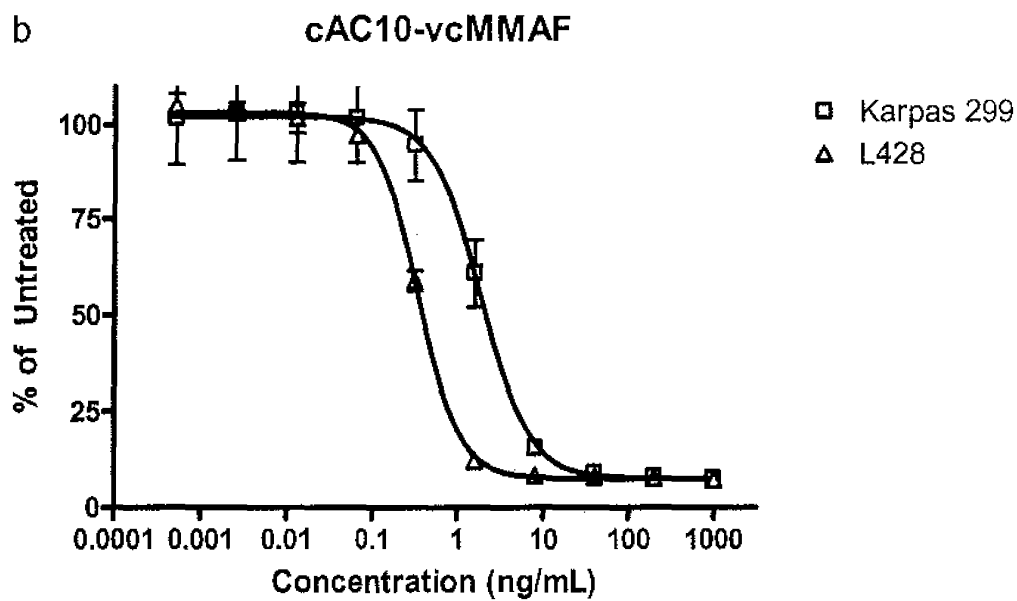


Fig. 4b

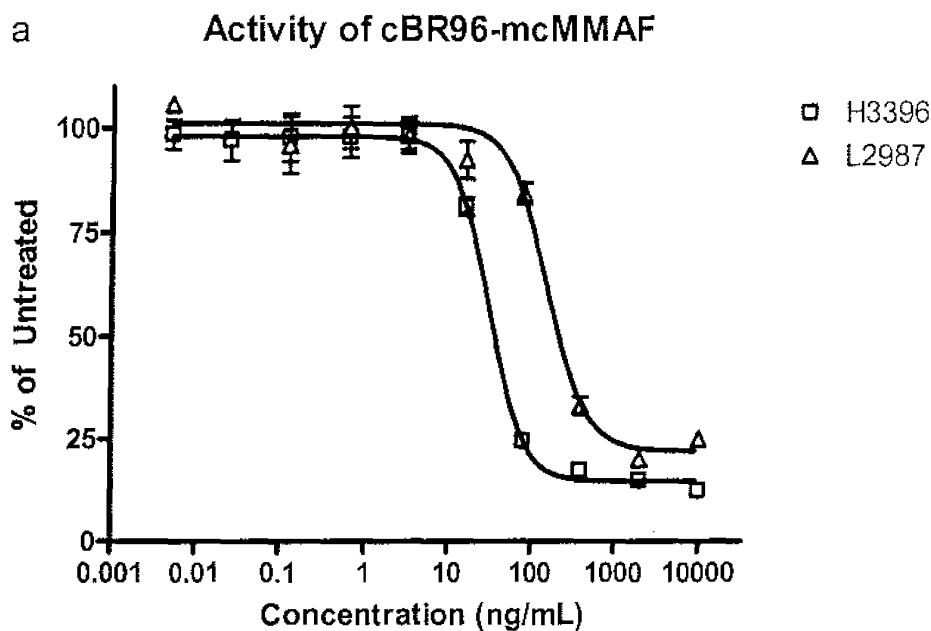


Fig. 5a

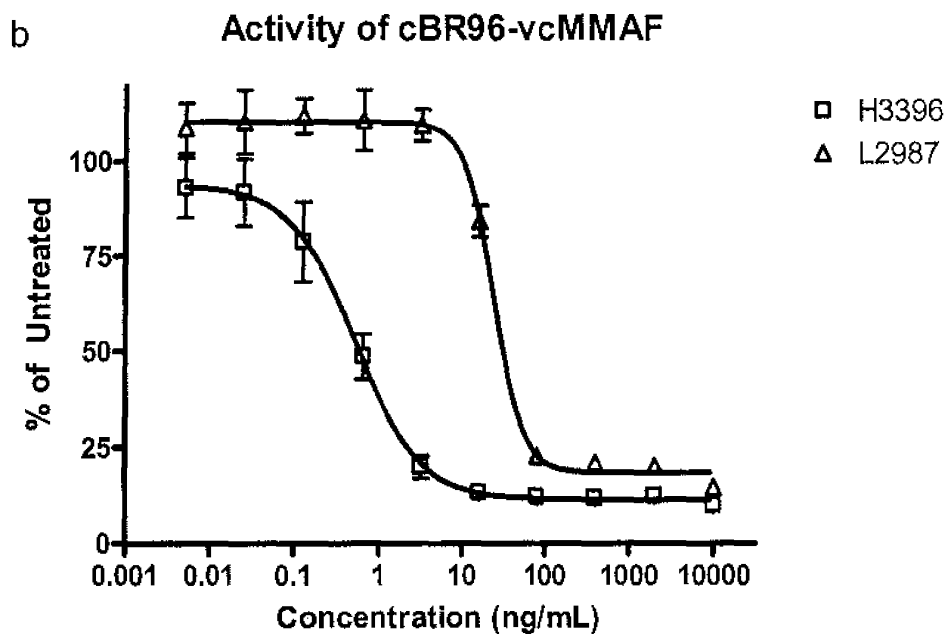


Fig. 5b

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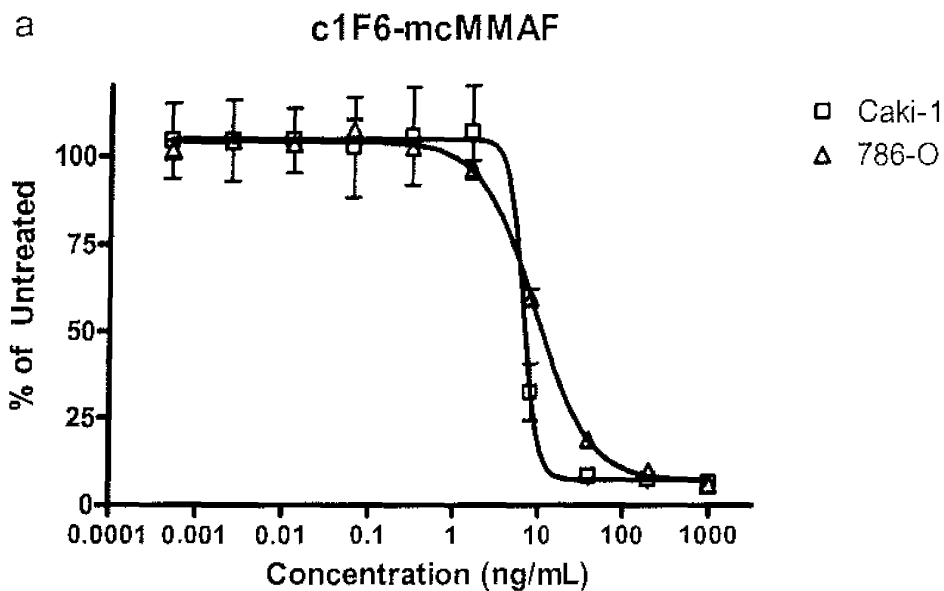


Fig. 6a

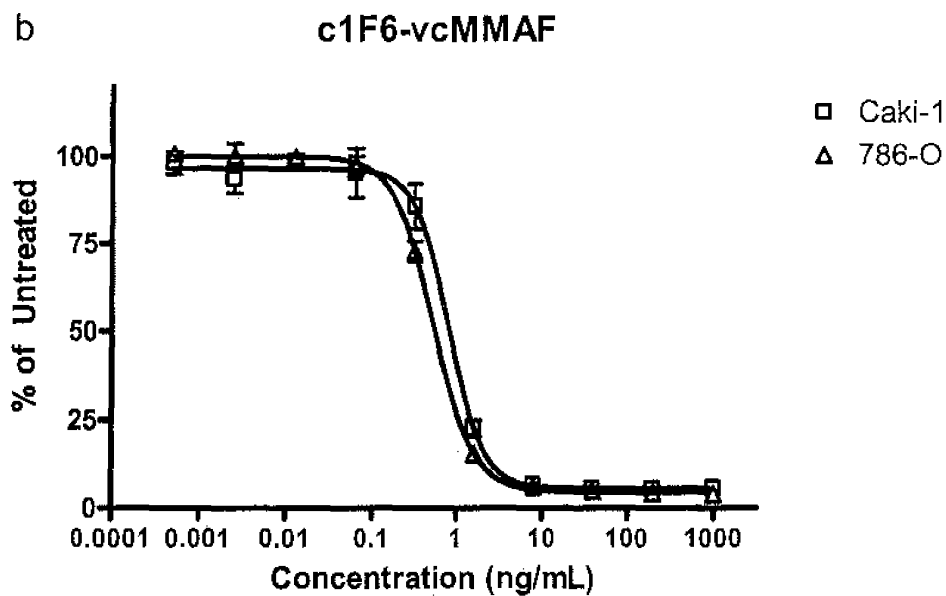


Fig. 6b

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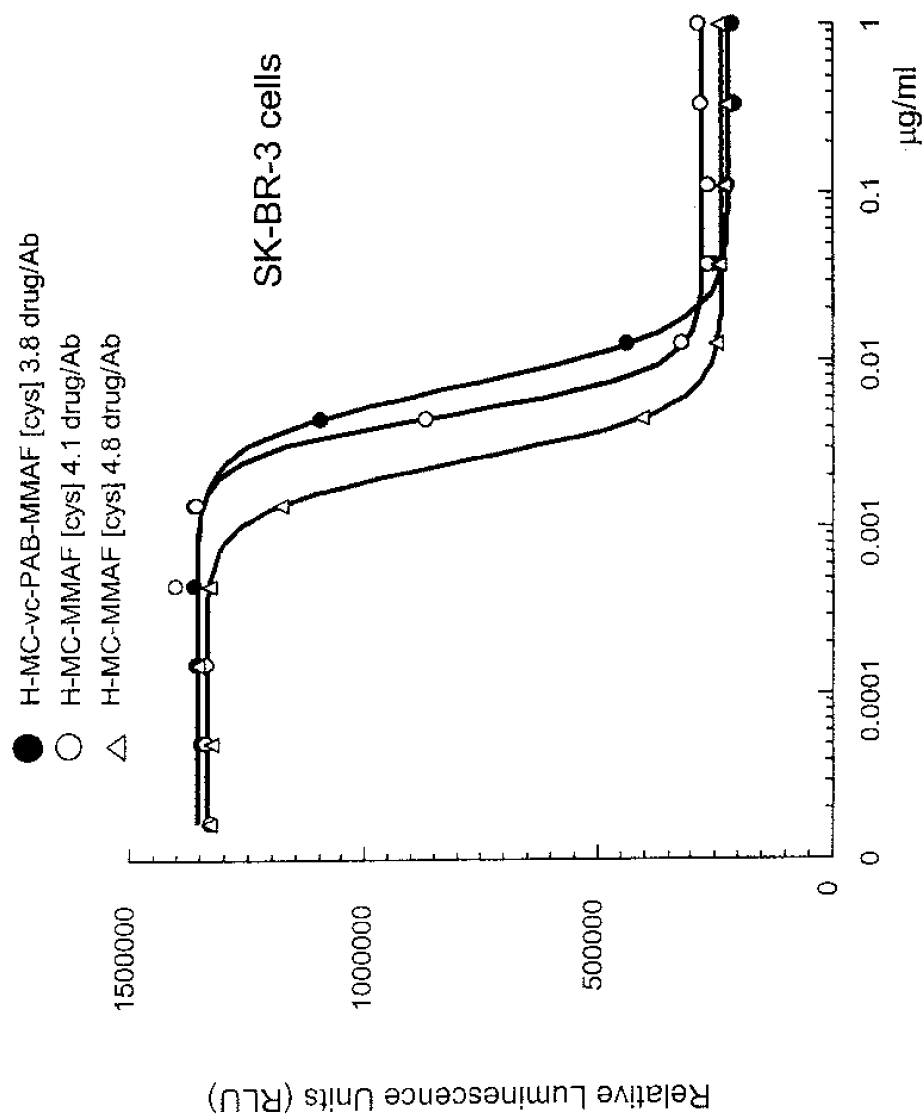


Fig. 7

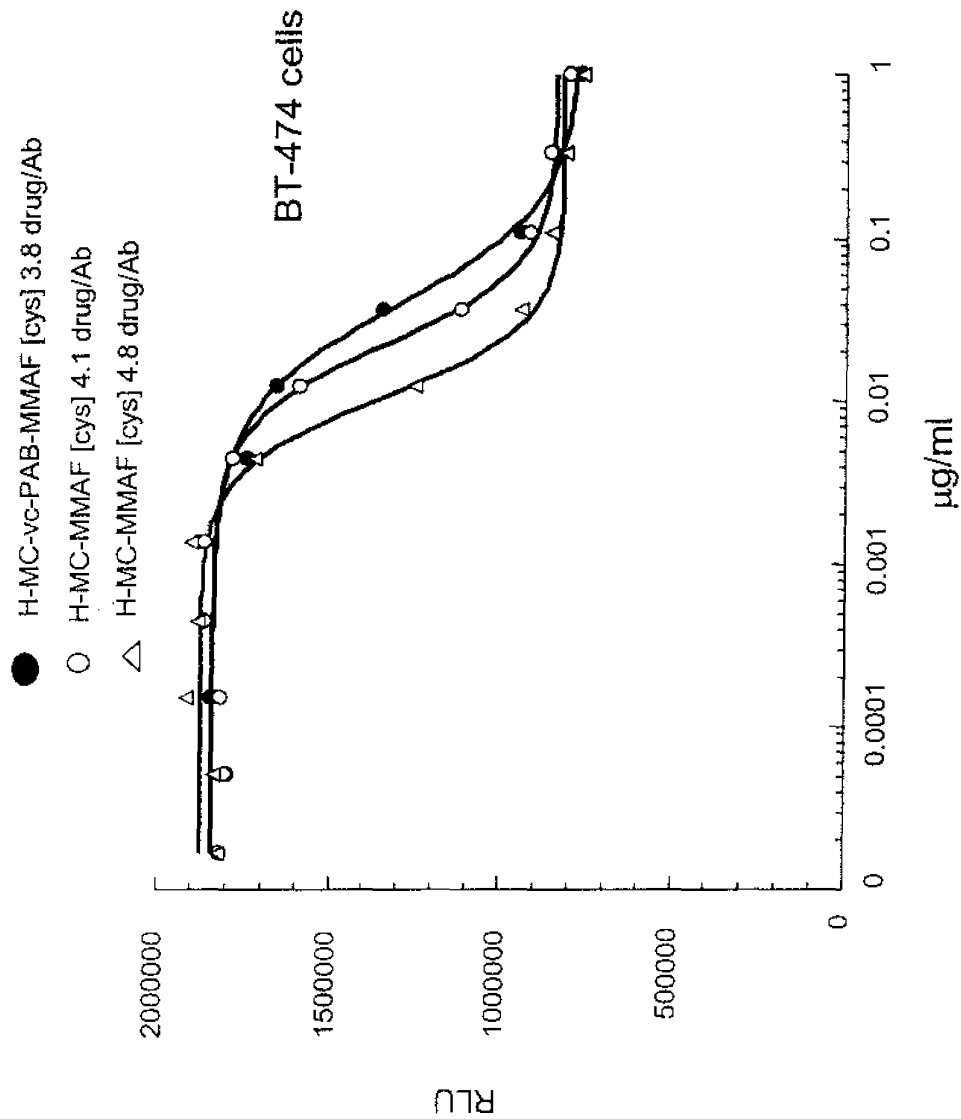


Fig. 8

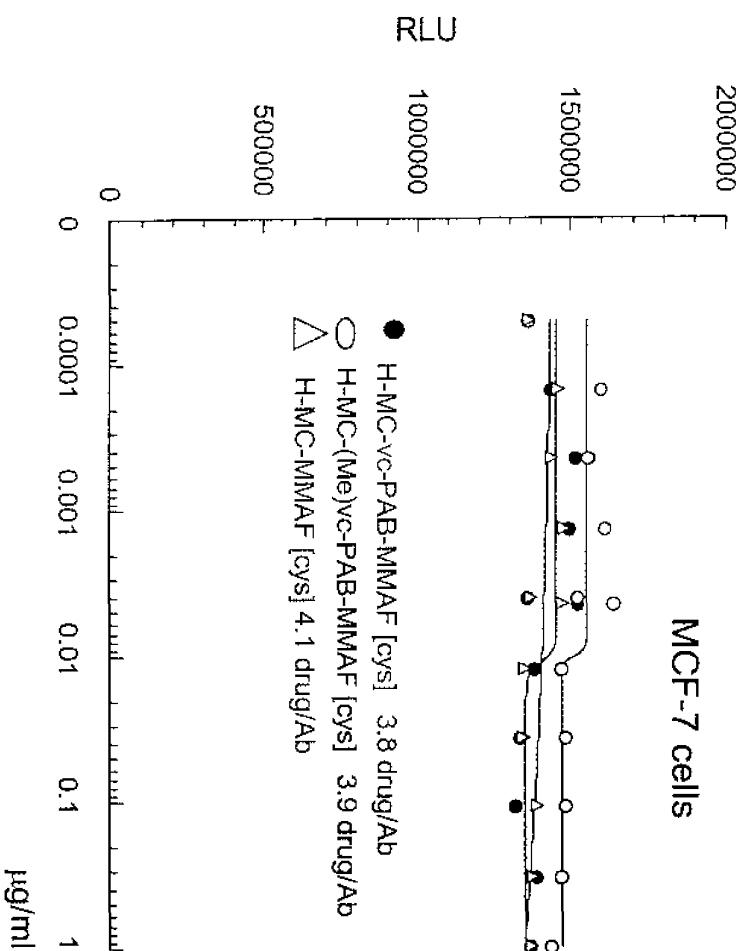


Fig. 9

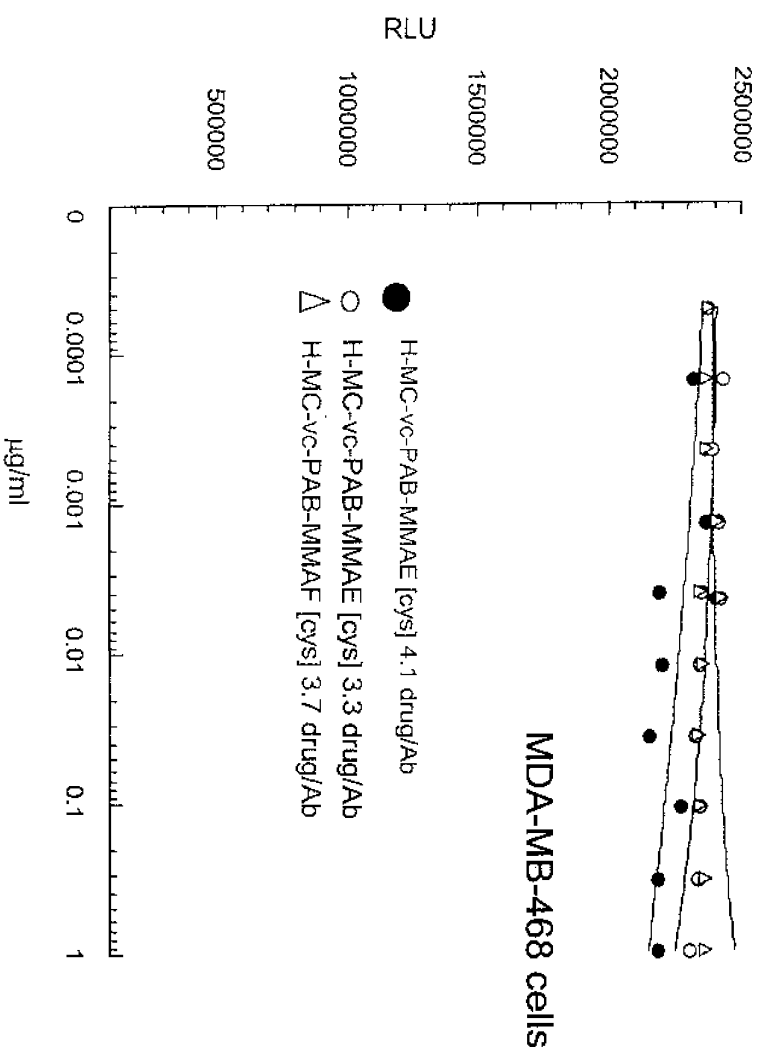


Fig. 10

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Appx77

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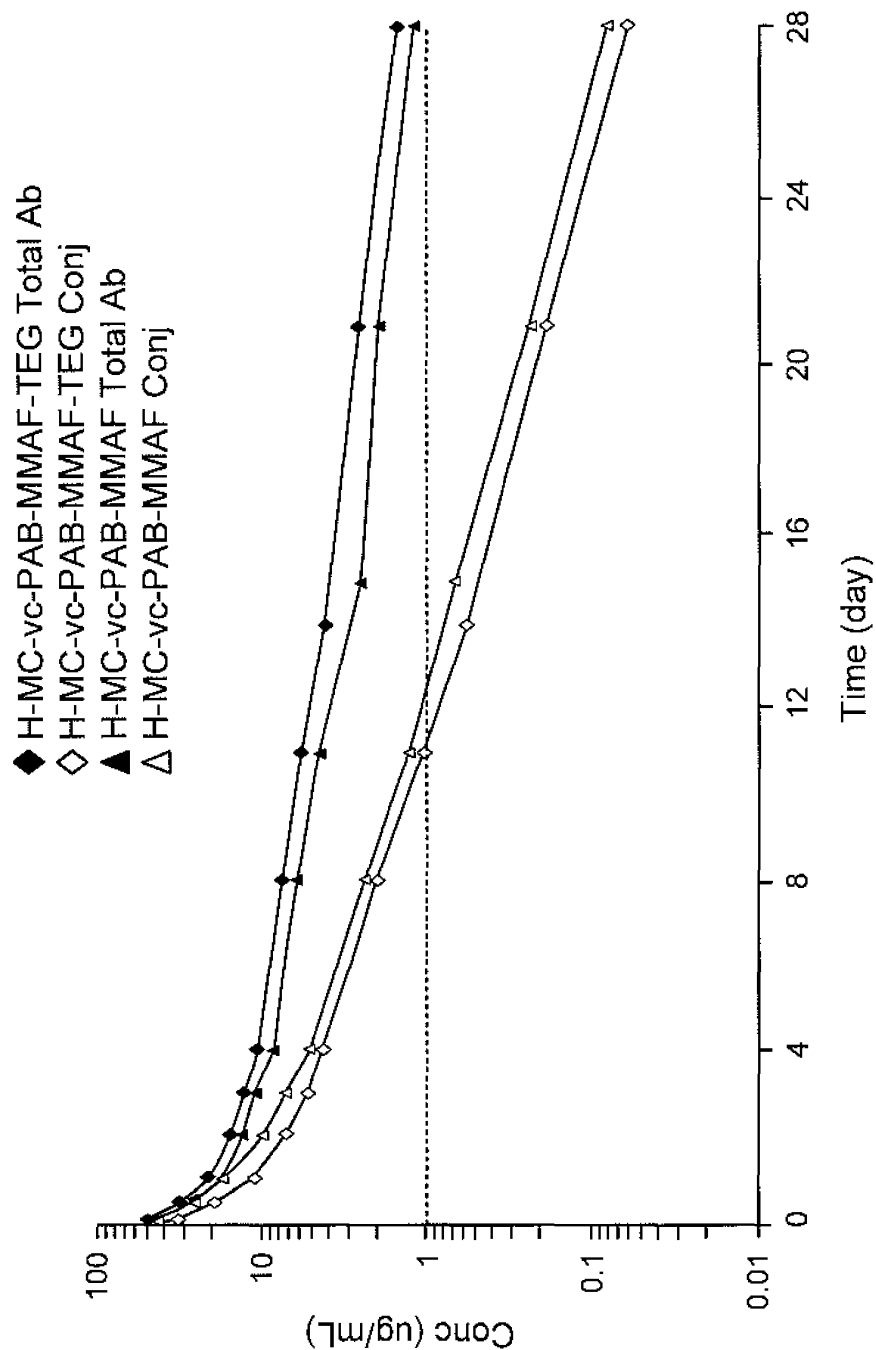


FIG. 11

H-MC-vc-PAB-MMAE in Cynomolgus monkeys

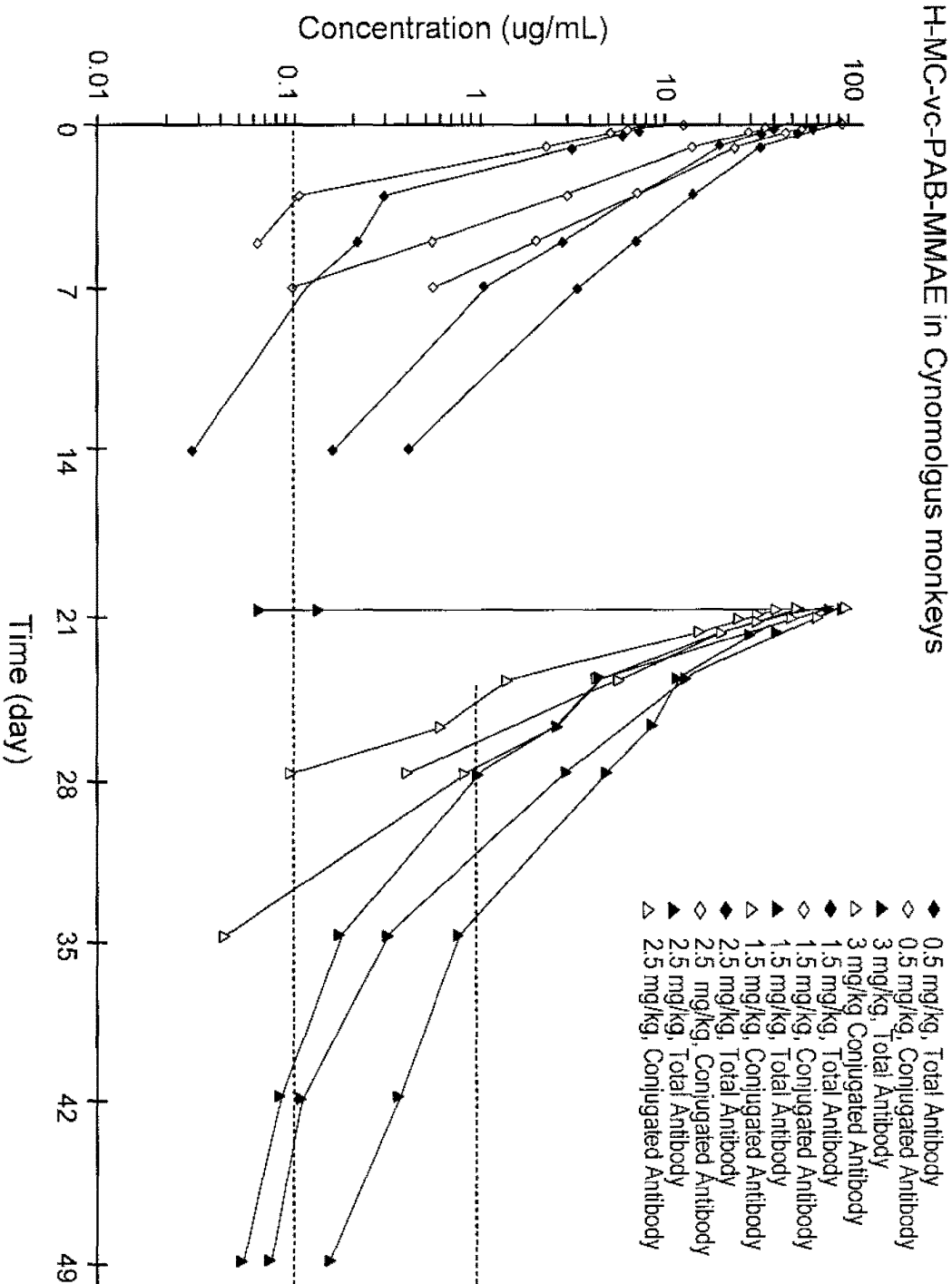


FIG. 12

DX-0001, Page 19 of 212

Appx79

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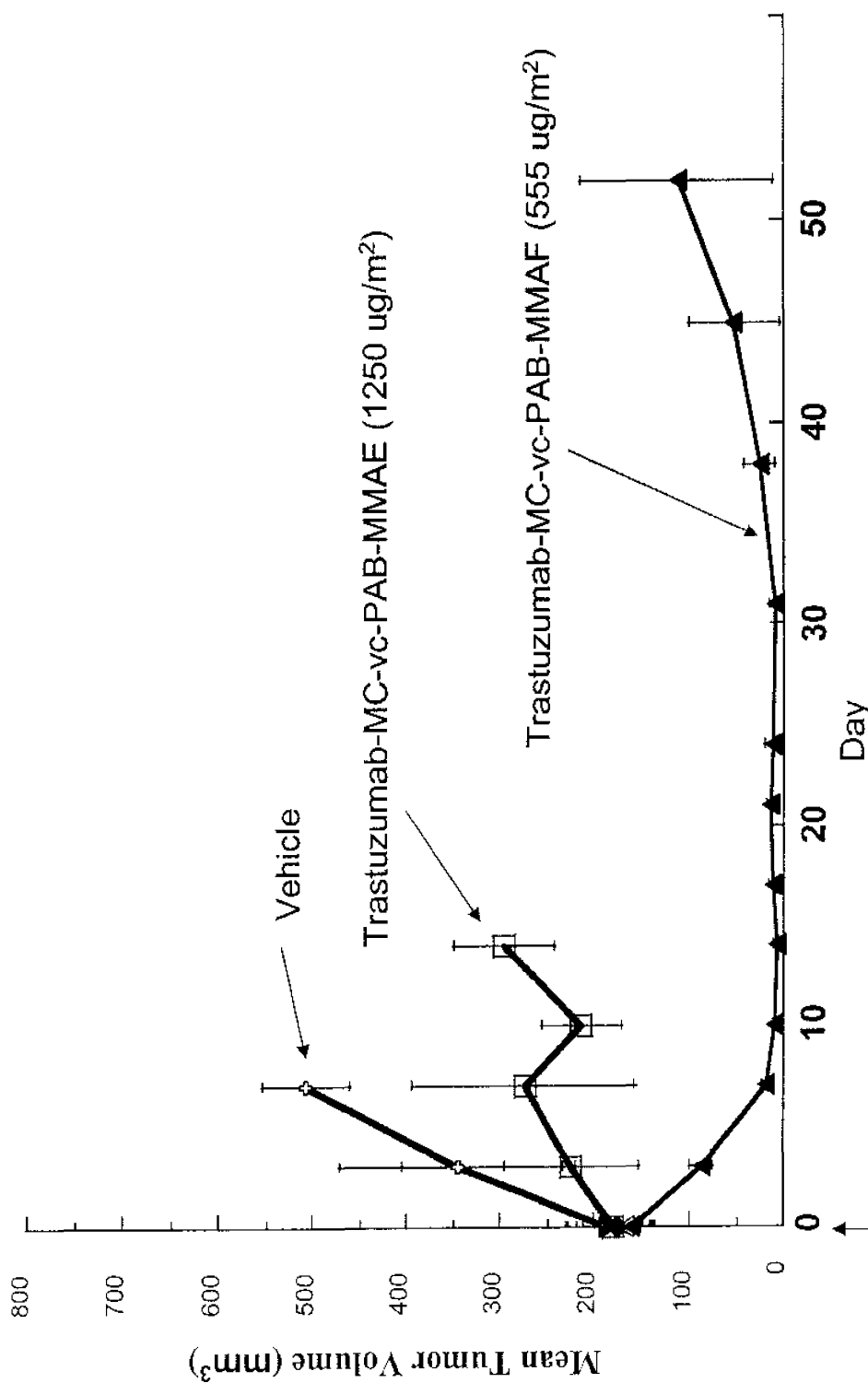


Fig. 13

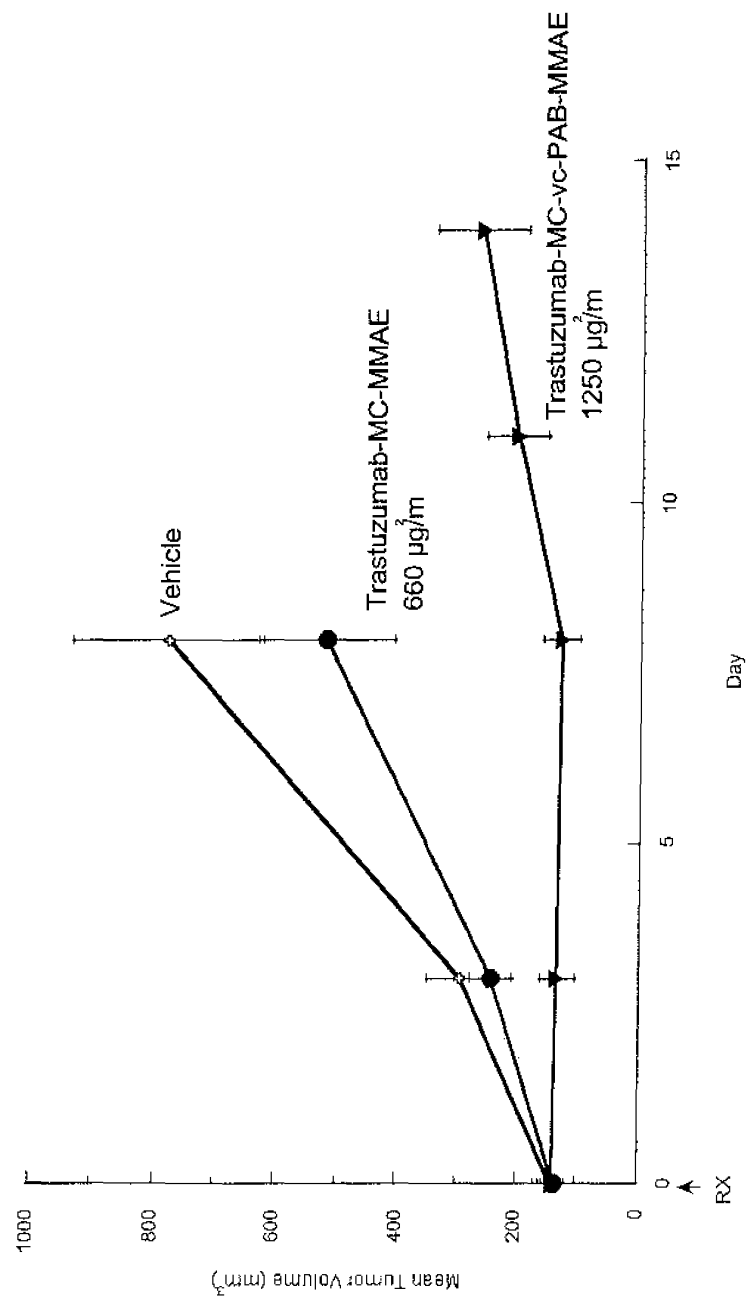


Fig. 14

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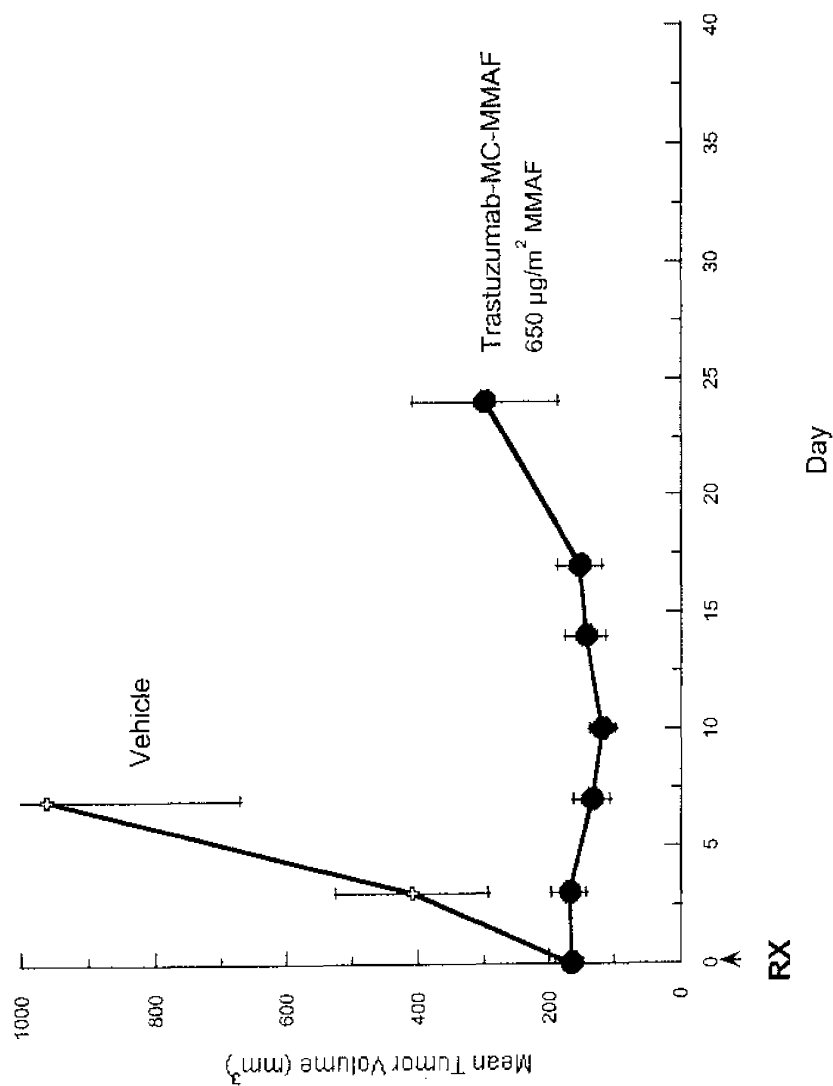


Fig. 15

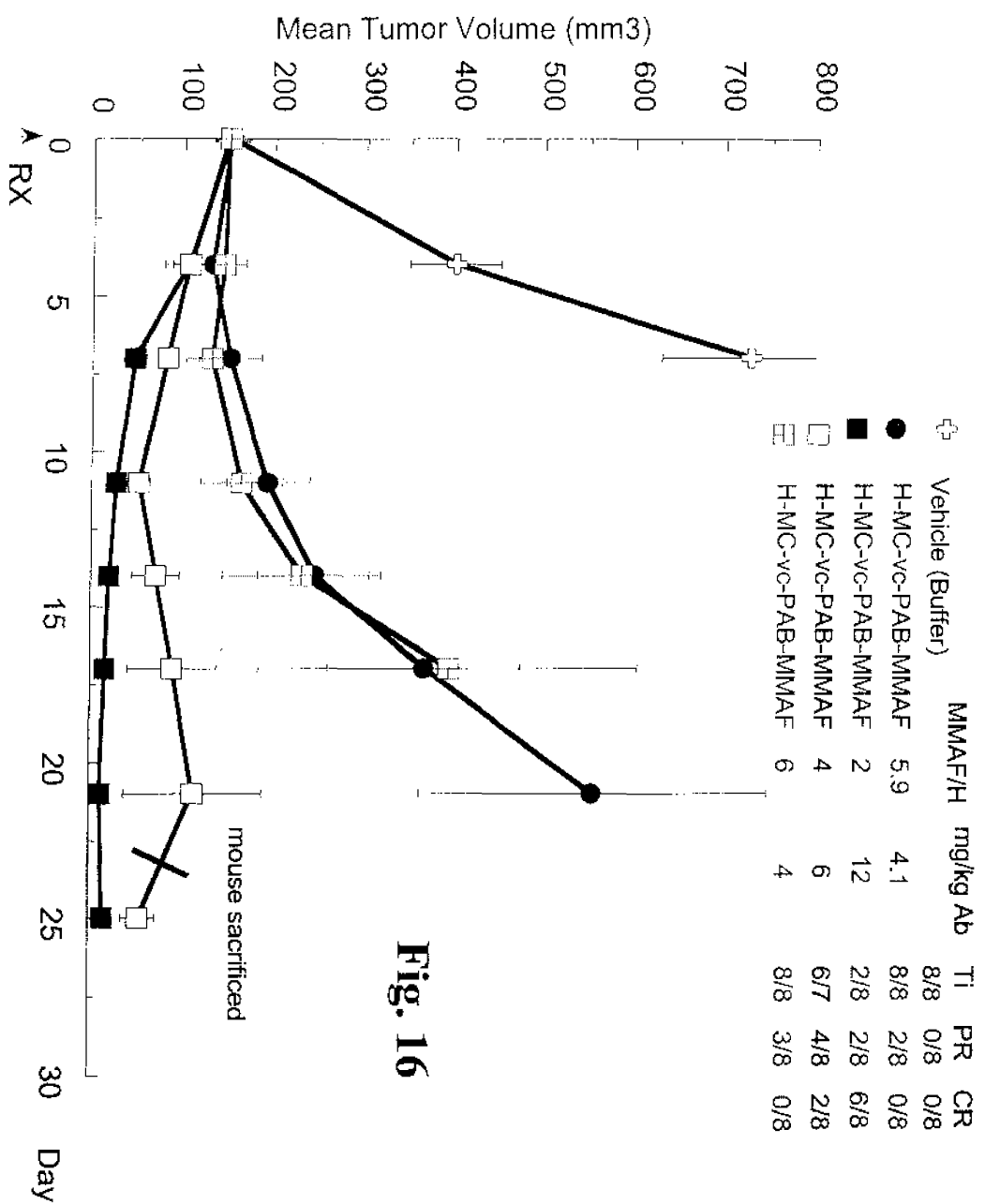


Fig. 16

DX-0001, Page 23 of 212

Appx83

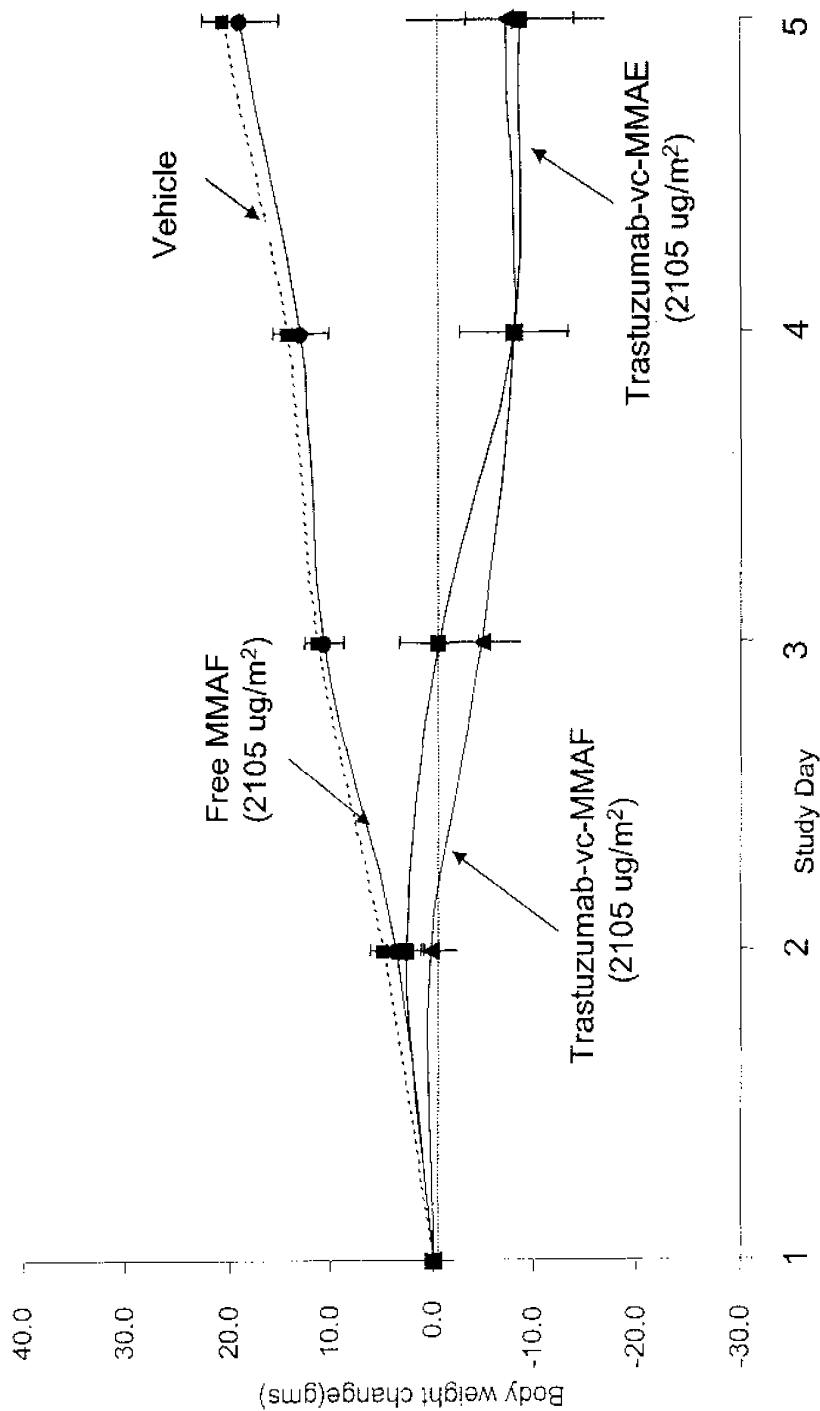


Fig. 17

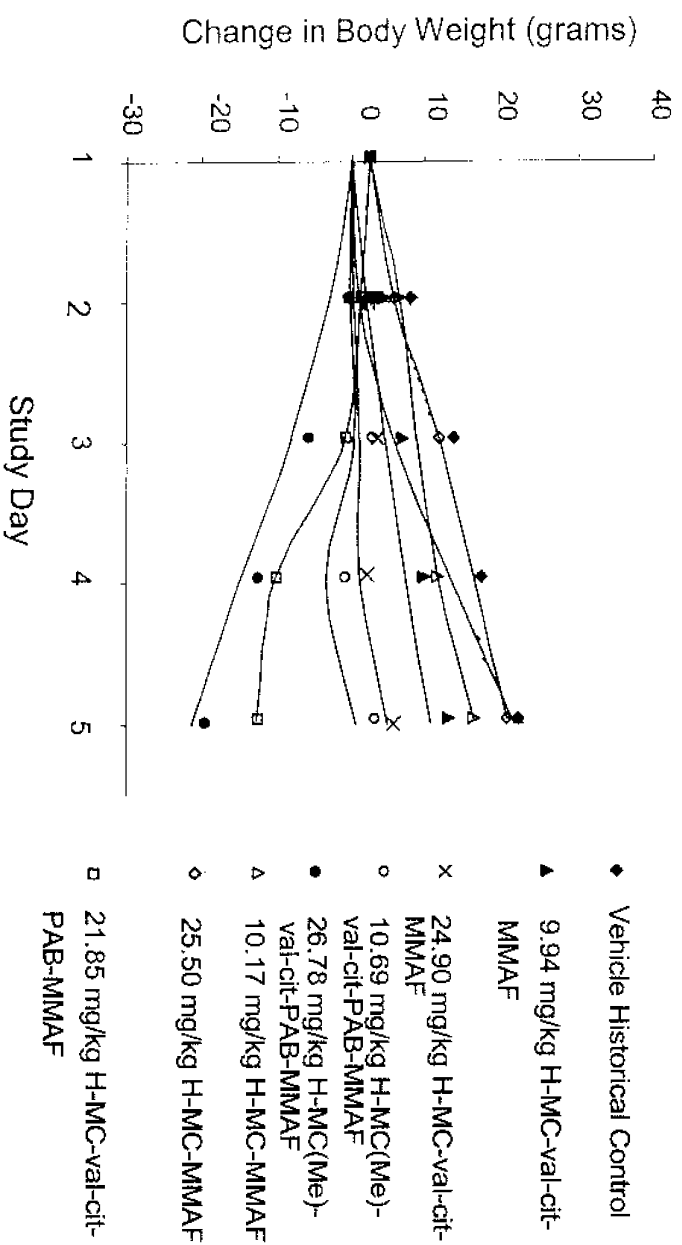


Fig. 18

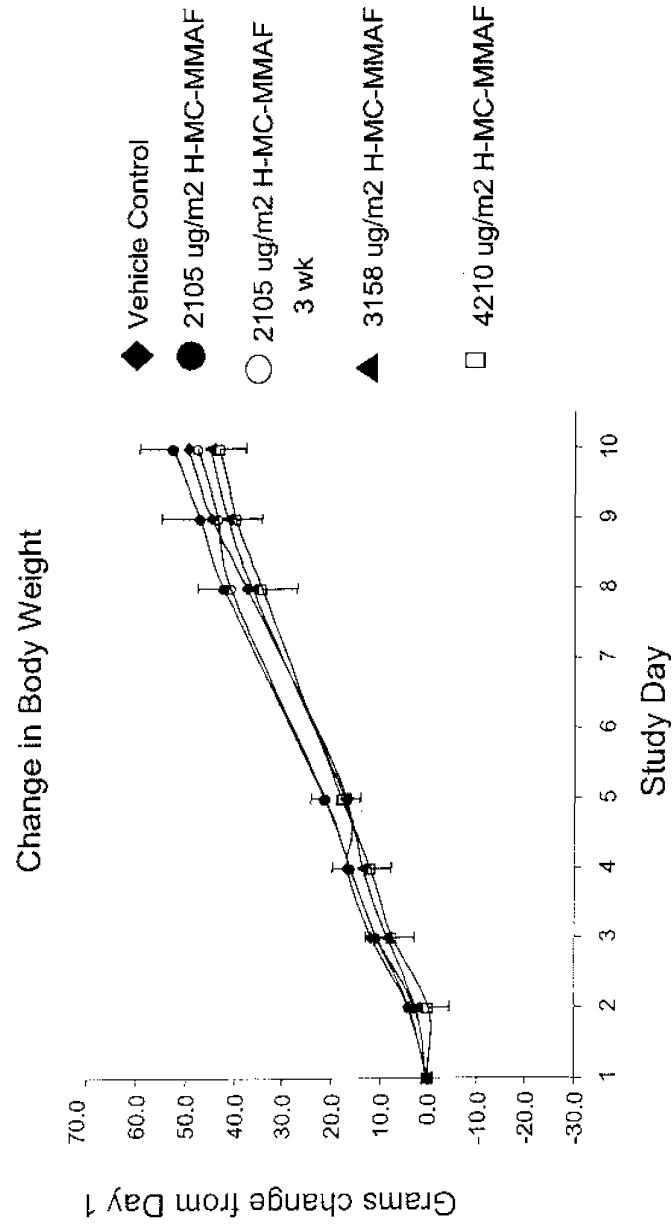


Fig. 19

Fig. 20

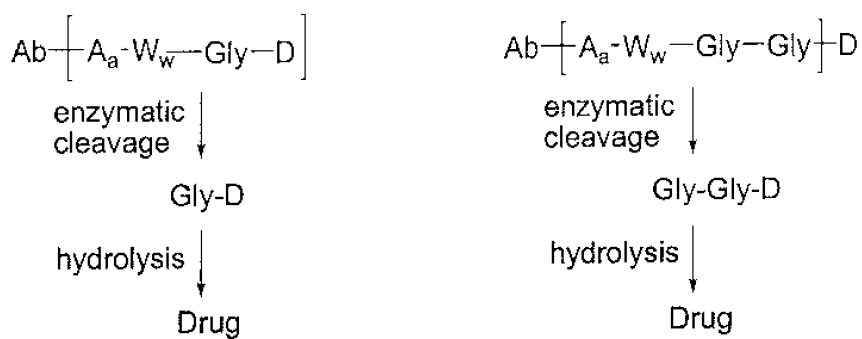


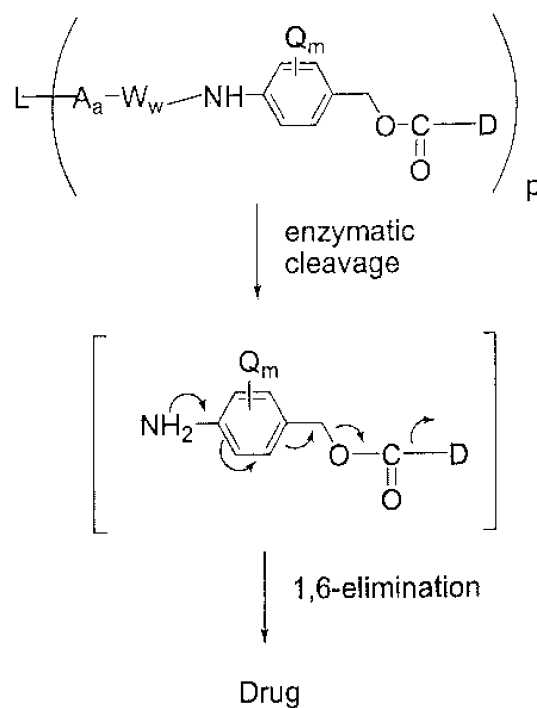
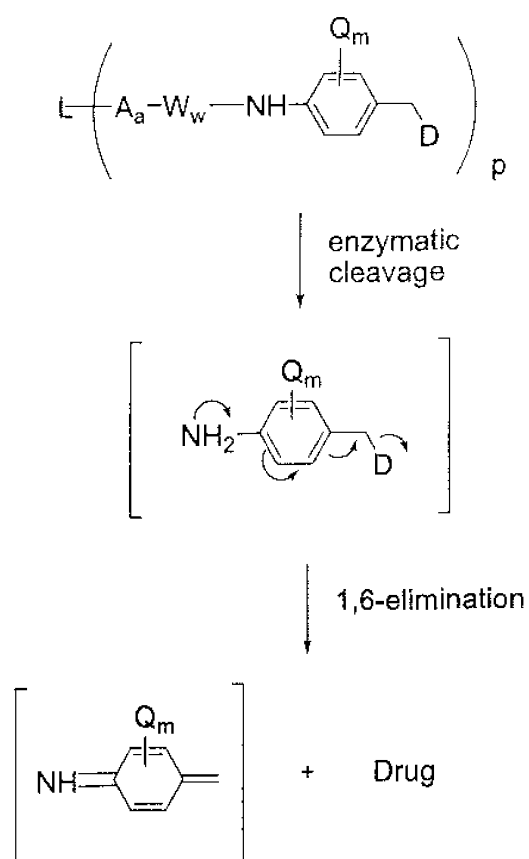
Fig. 21

Fig. 22



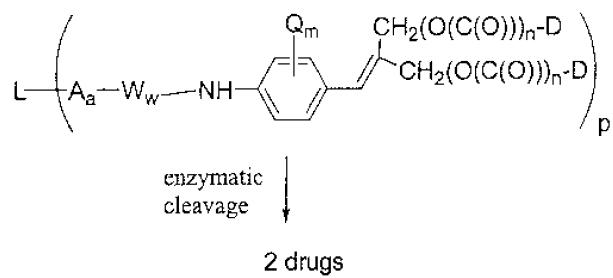
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Fig. 23



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Fig. 24

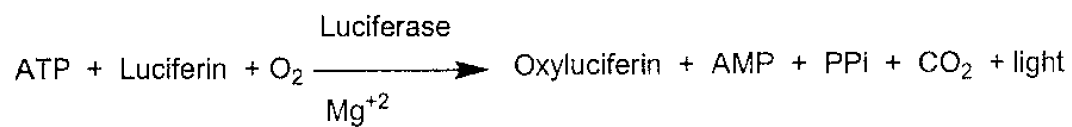
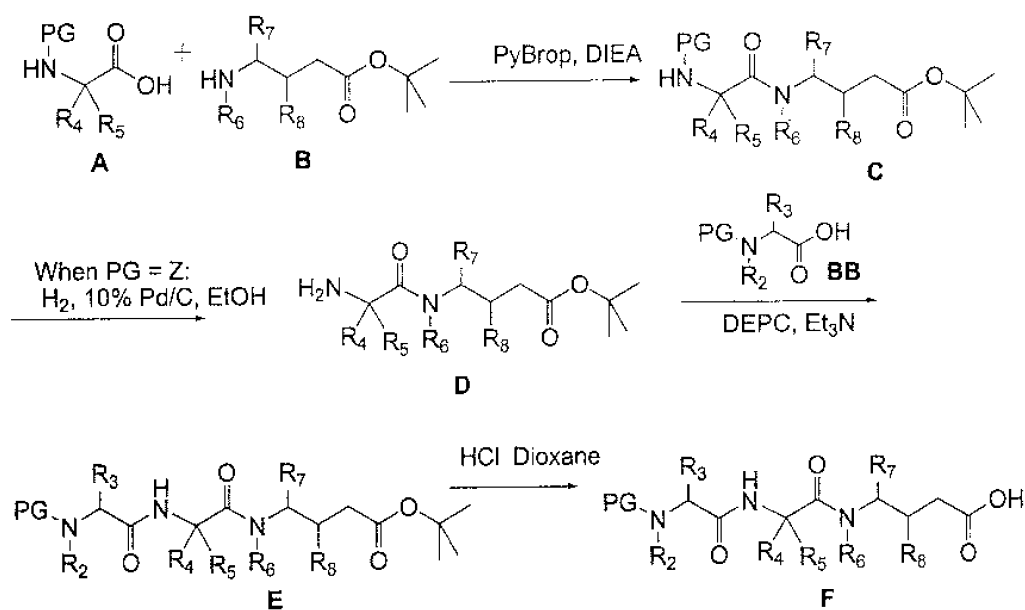


Fig. 25



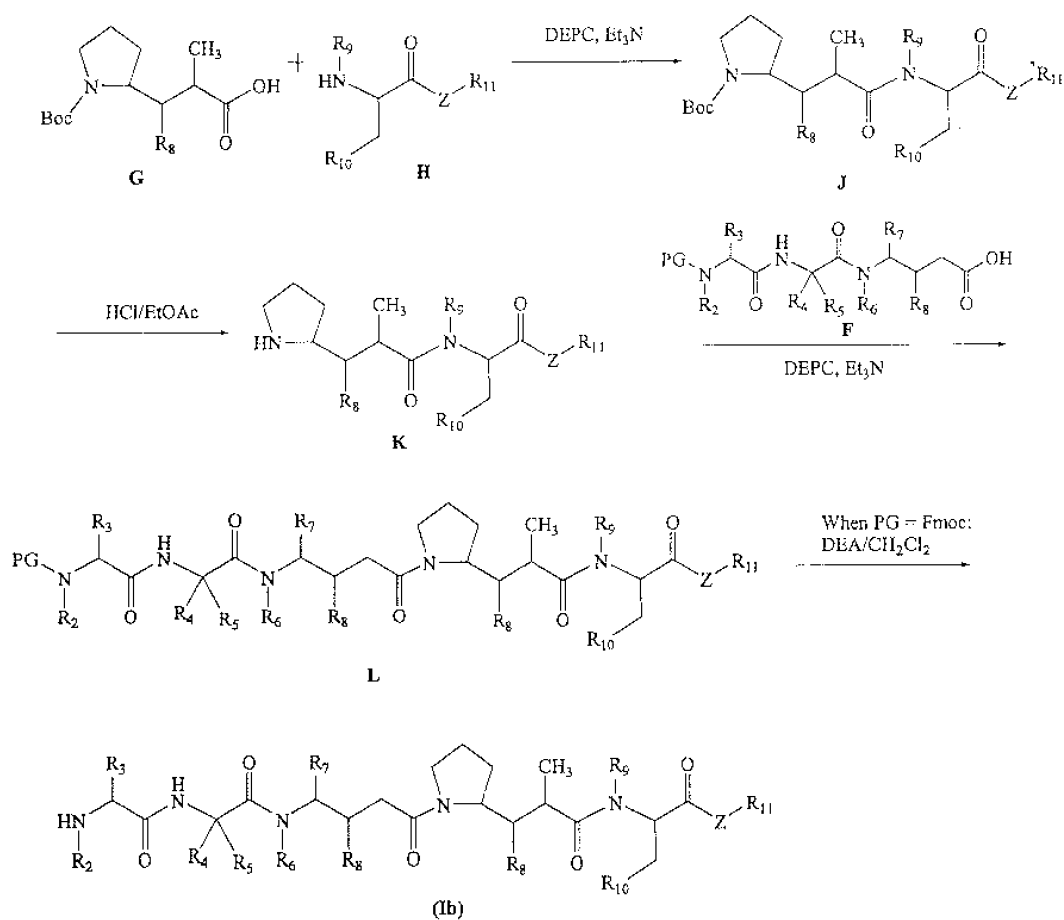
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Fig. 26



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Fig. 27

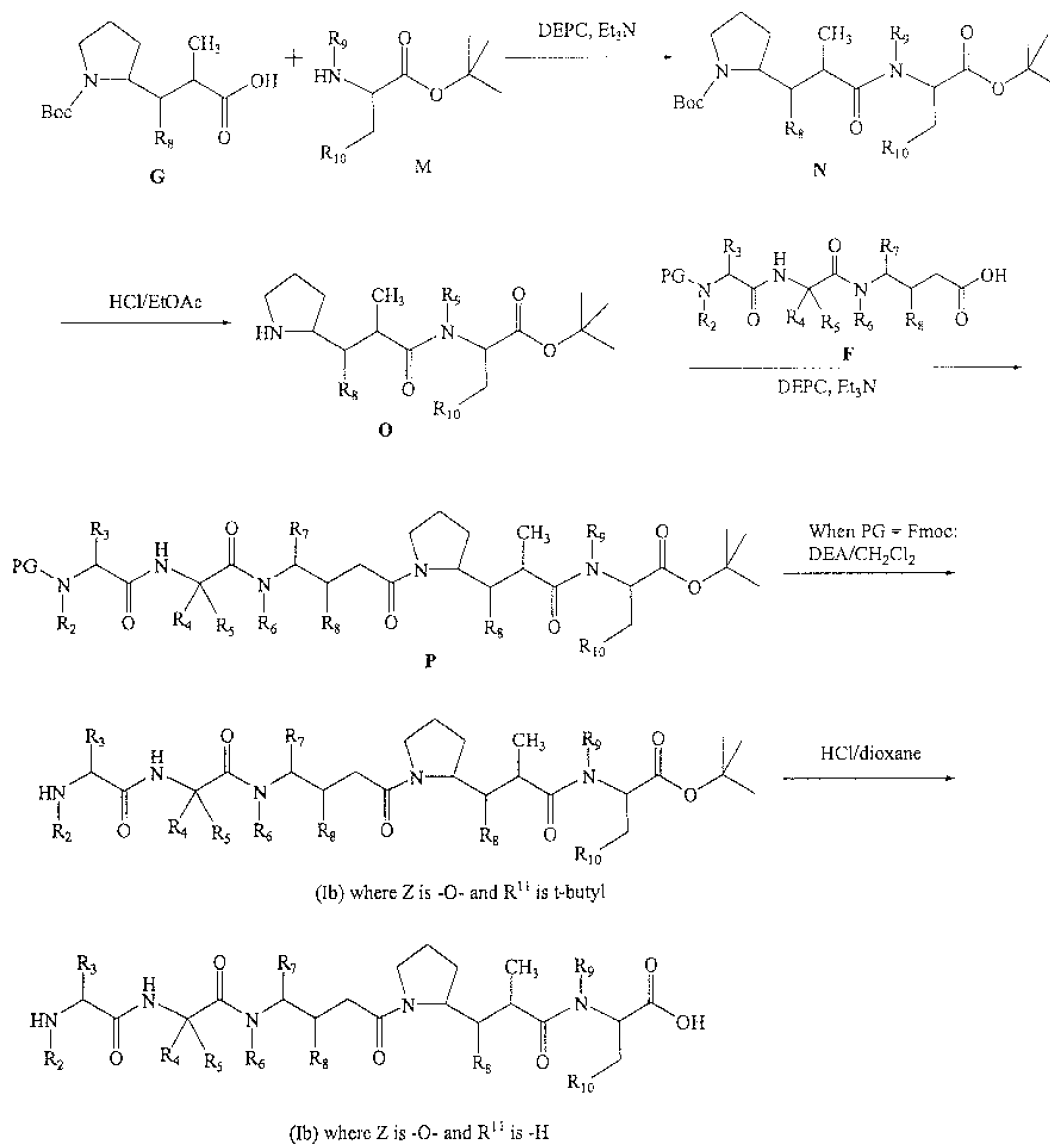


Fig. 28

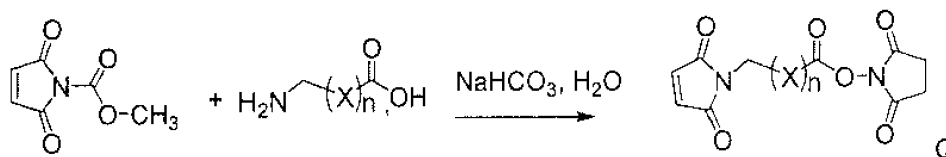
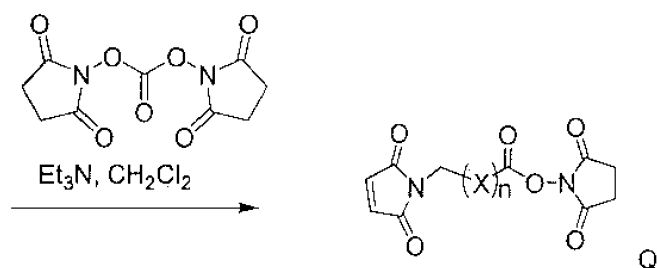
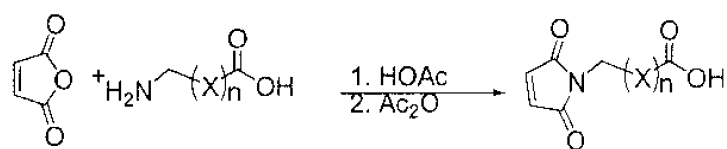
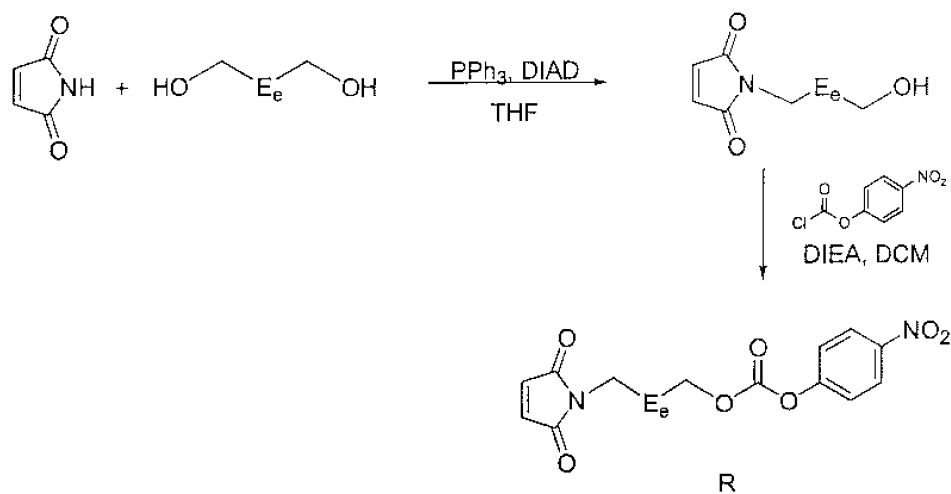


Fig. 29

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Fig. 30

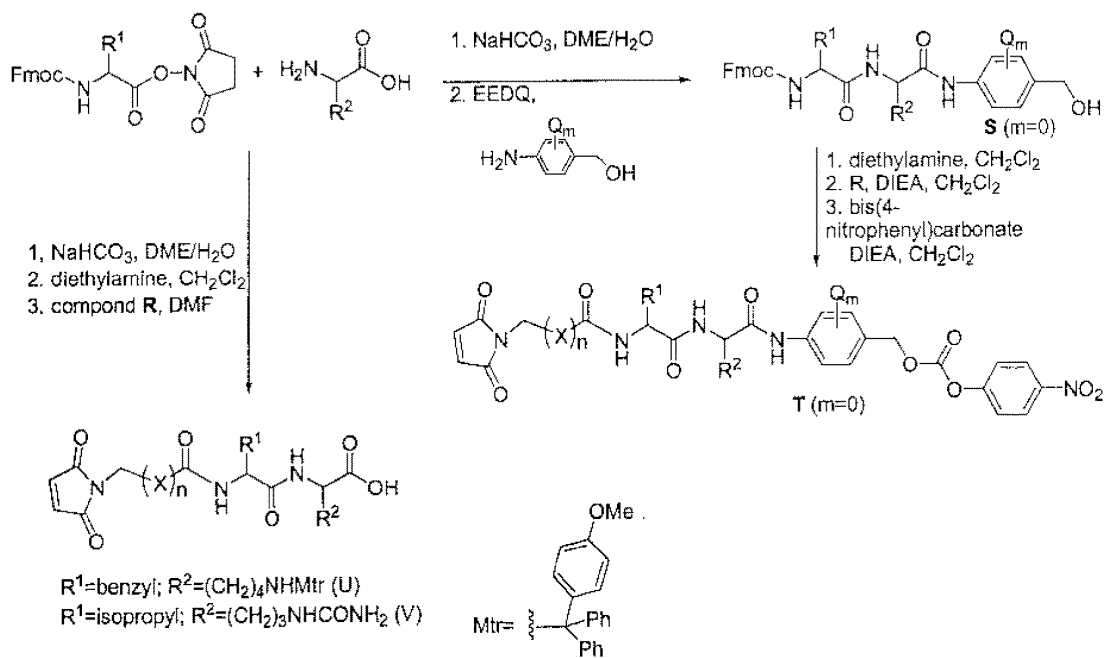


Fig. 31

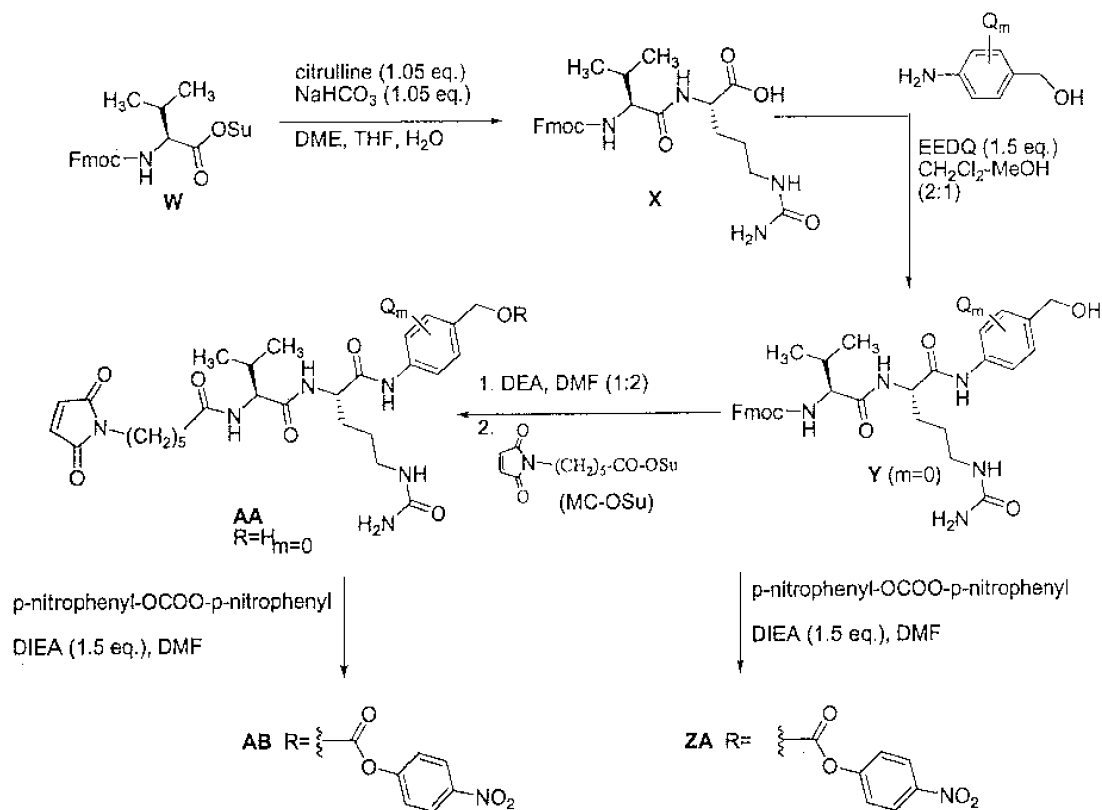


Fig. 32

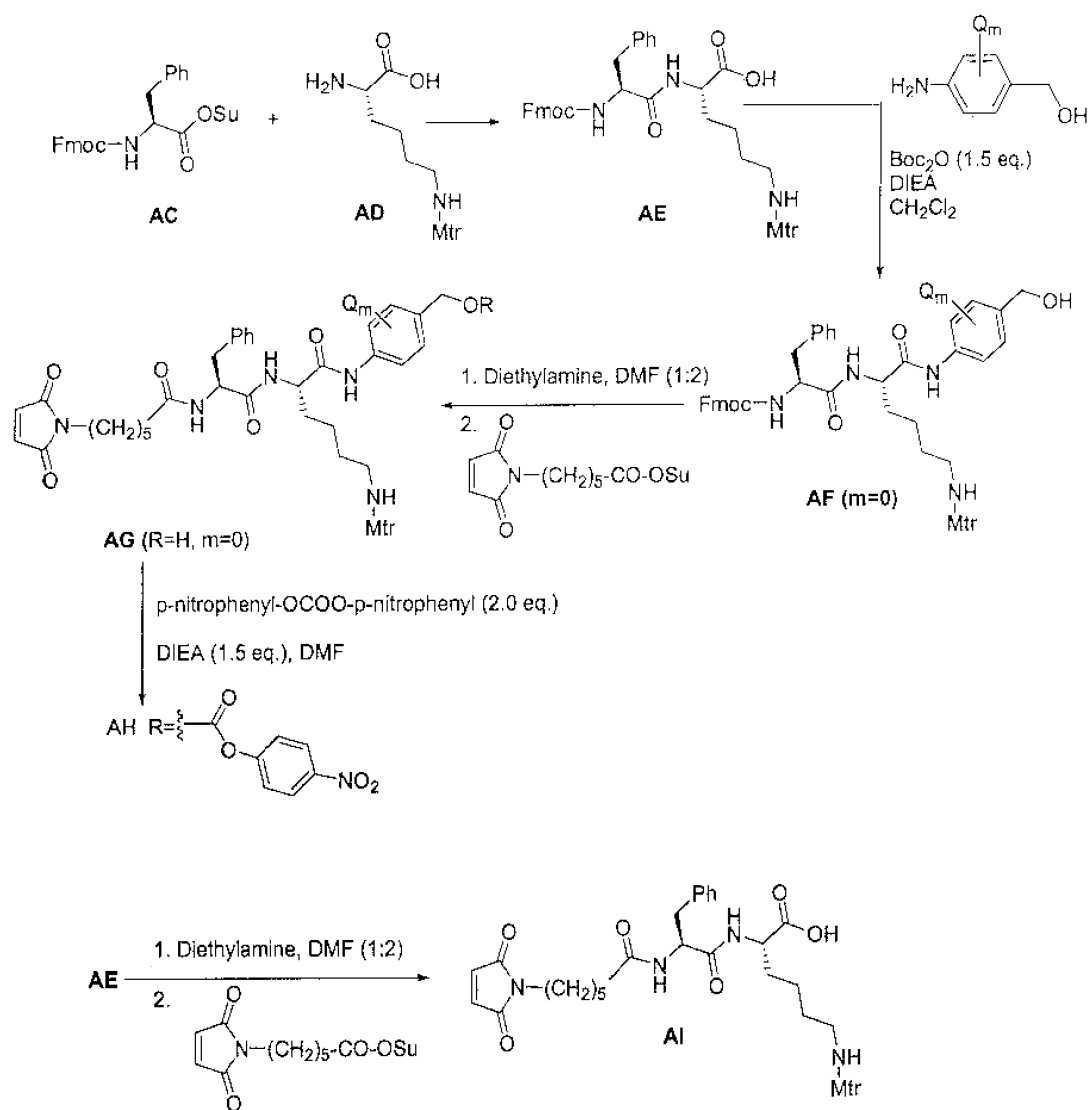


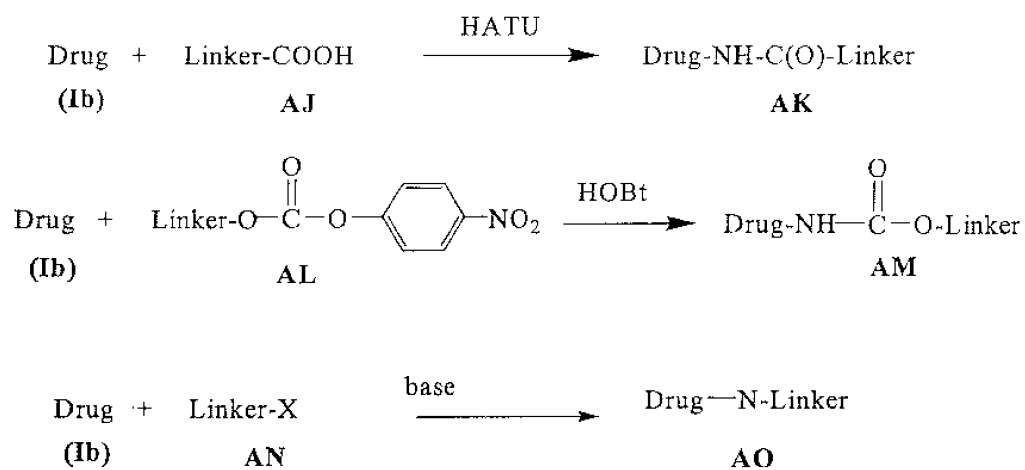
Fig. 33

Fig. 34

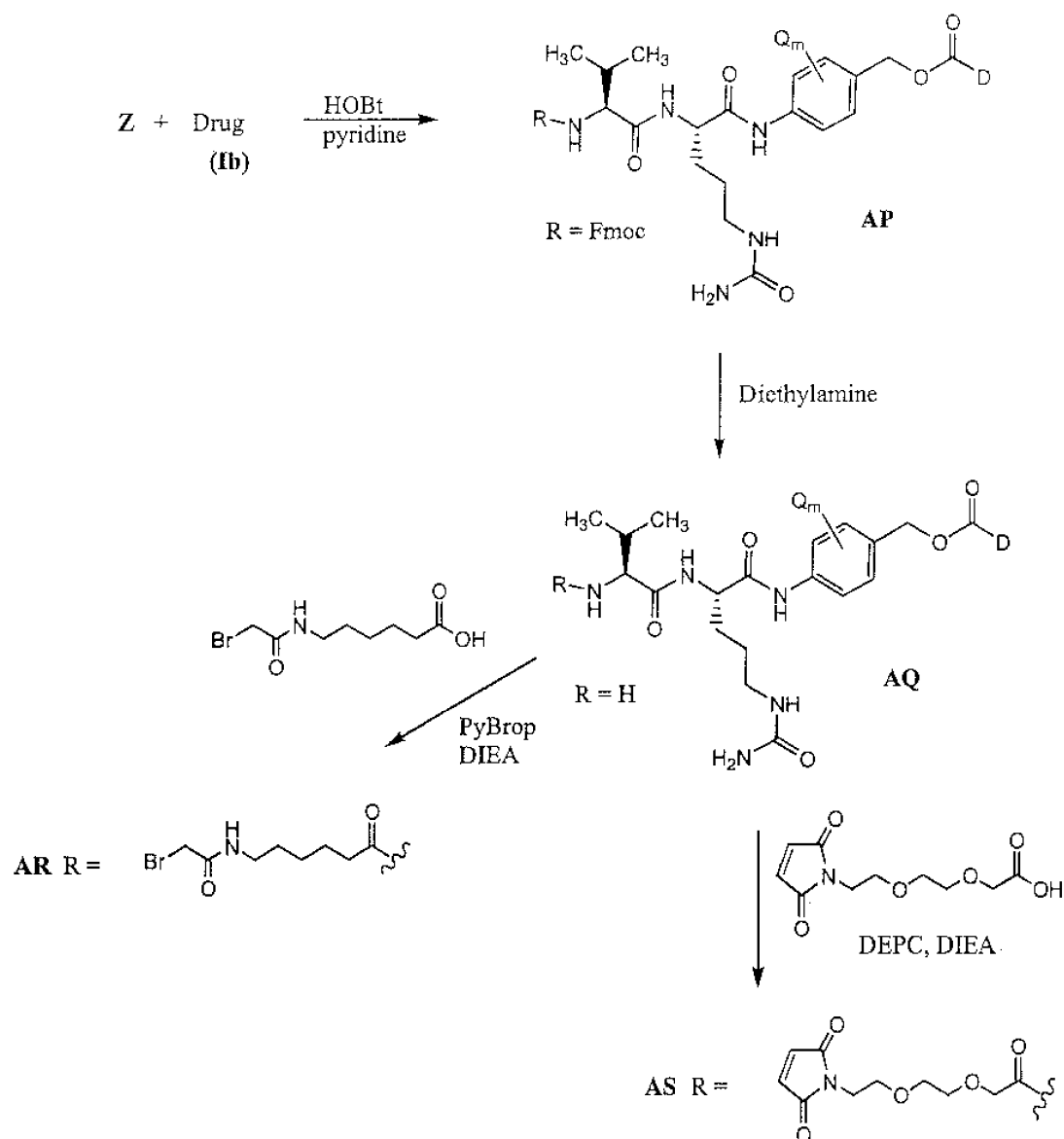
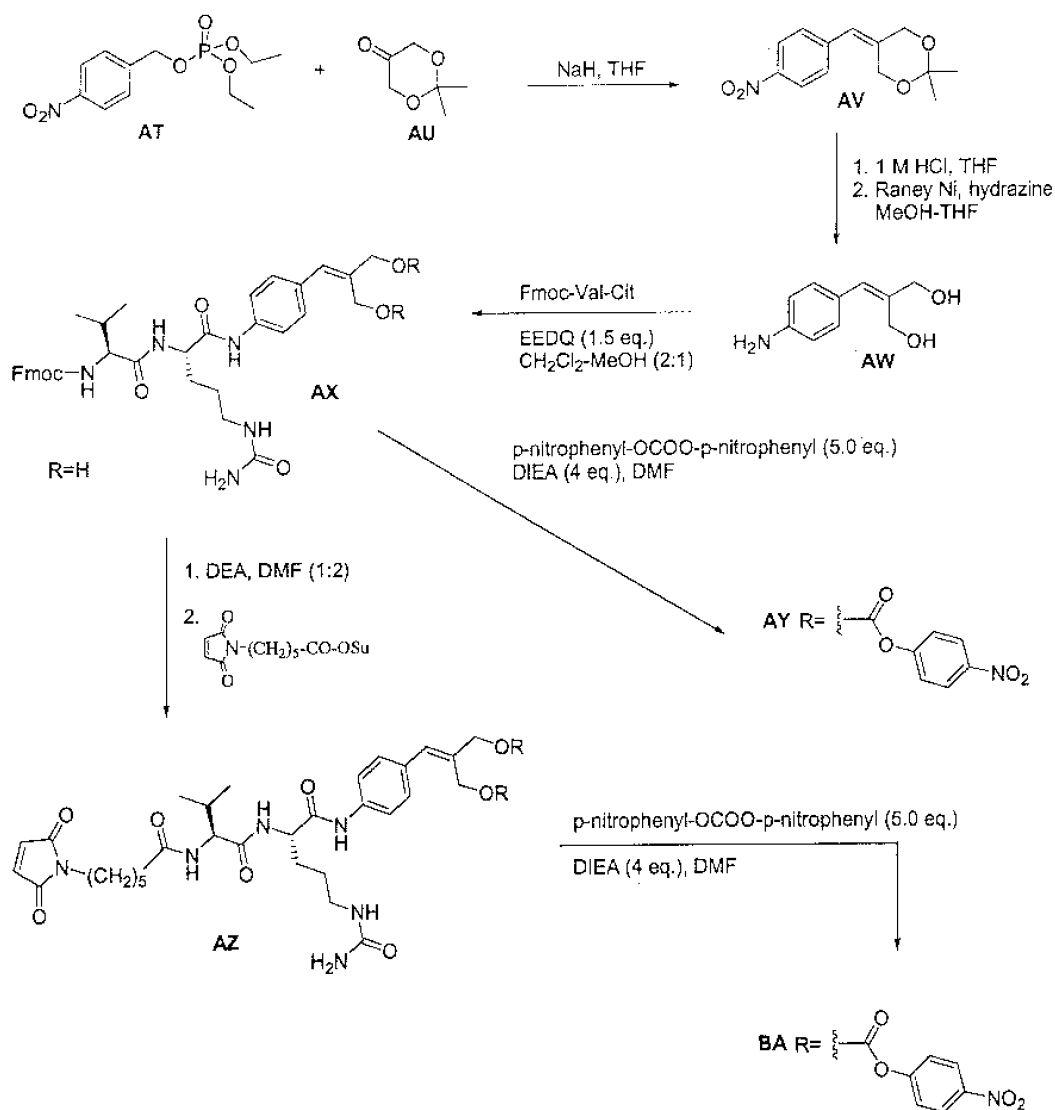


Fig. 35



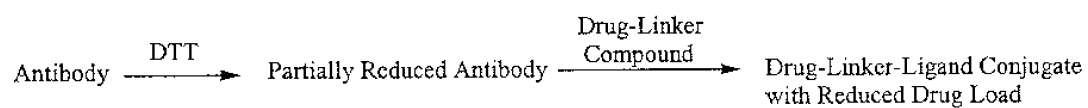
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Fig. 36



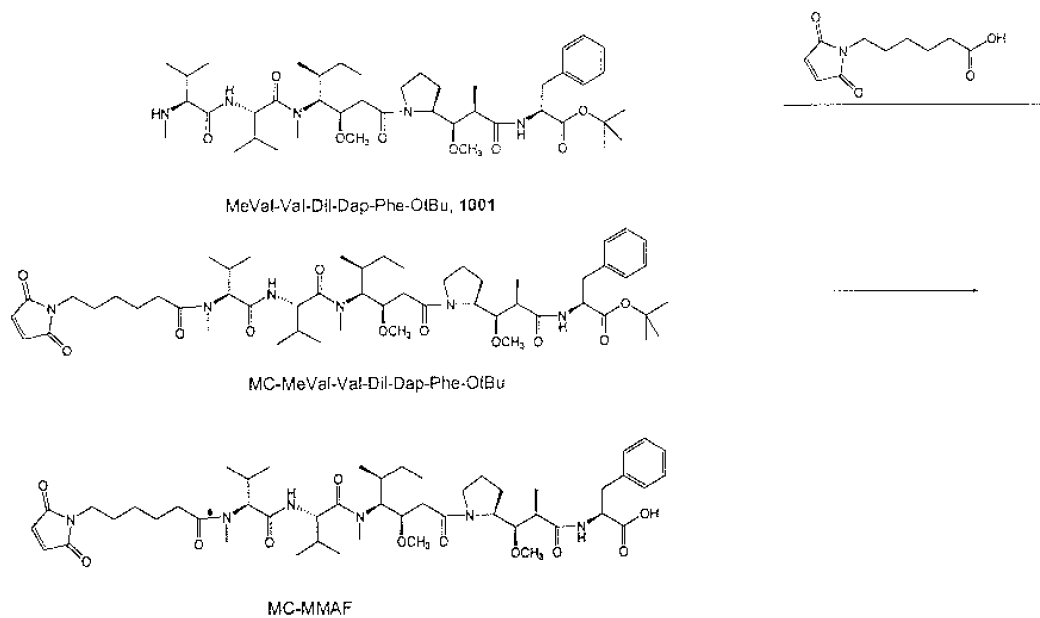
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Fig. 37



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Fig. 38

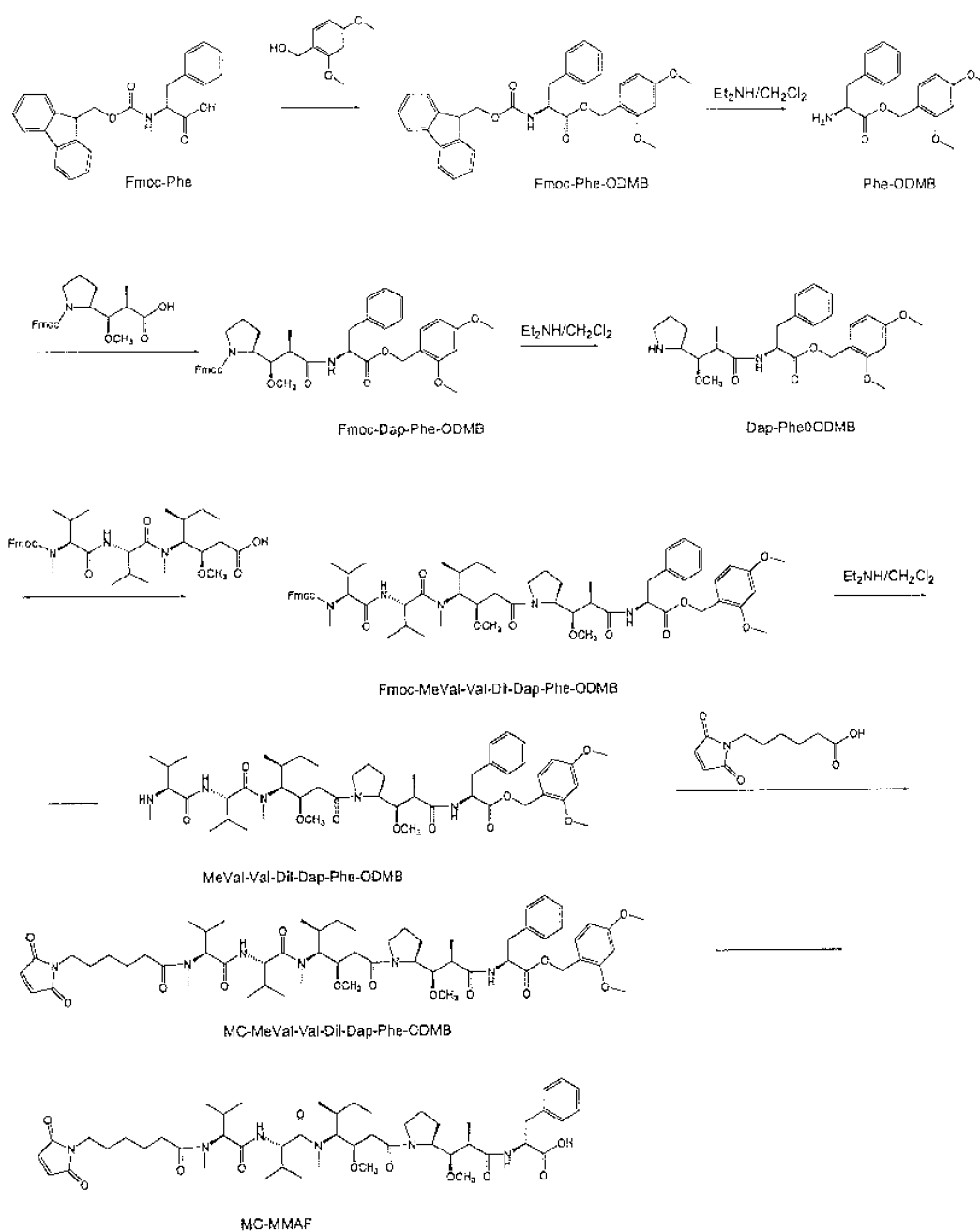
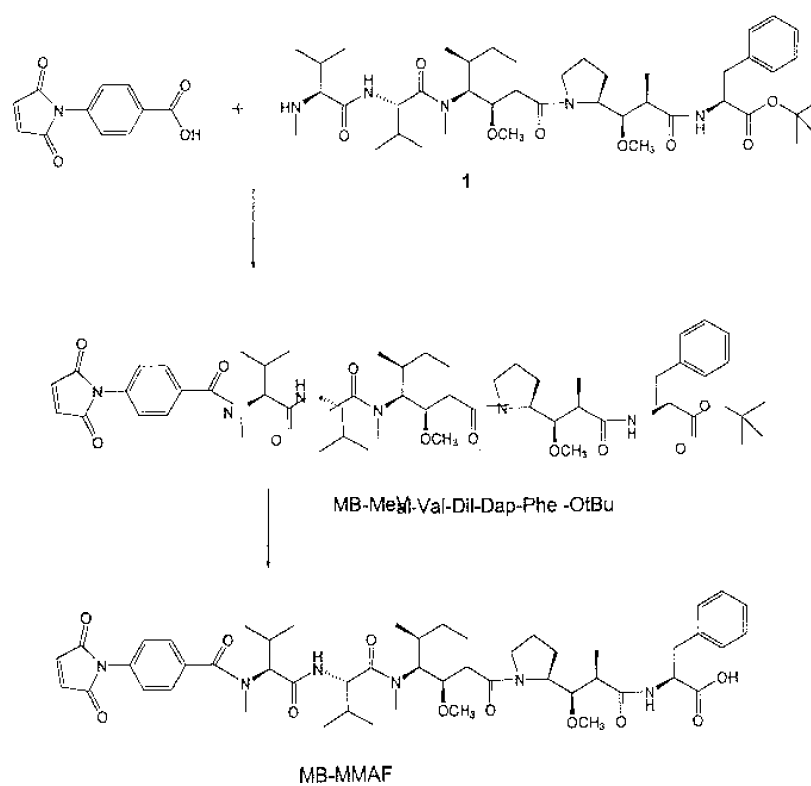


Fig. 39



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1

**MONOMETHYLVALINE COMPOUNDS
CAPABLE OF CONJUGATION TO LIGANDS****1. CROSS-REFERENCES TO RELATED
APPLICATIONS**

This application is a continuation of U.S. patent application Ser. No. 15/811,190 filed Nov. 13, 2017, which is a continuation of U.S. patent application Ser. No. 15/188,843 filed Jun. 21, 2016, which is a continuation of U.S. patent application Ser. No. 14/194,106 filed Feb. 28, 2014, which is a continuation of U.S. patent application Ser. No. 13/098,391 filed Apr. 29, 2011 (now U.S. Pat. No. 8,703,714), which is a continuation of U.S. patent application Ser. No. 11/833,954 filed Aug. 3, 2007 (now U.S. Pat. No. 7,994,135), which is a divisional of U.S. patent application Ser. No. 10/983,340 filed Nov. 5, 2004 (now U.S. Pat. No. 7,498,298), which is an application claiming the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Patent Application No. 60/518,534, filed Nov. 6, 2003, and U.S. Provisional Patent Application No. 60/557,116, filed Mar. 26, 2004, and U.S. Provisional Patent Application No. 60/598,899, filed Aug. 4, 2004, and U.S. Provisional Patent Application No. 60/622,455, filed Oct. 27, 2004, each of which is incorporated herein by reference in its entirety.

2. JOINT RESEARCH AGREEMENT

Some of the subject matter in this application was made by or on behalf of Seattle Genetics, Inc. and Genentech, Inc. as a result of activities undertaken within the scope of a joint research agreement effective on or before the date the claimed invention was made.

**3. STATEMENT AS TO RIGHTS TO
INVENTIONS MADE UNDER FEDERALLY
SPONSORED RESEARCH AND
DEVELOPMENT**

NOT APPLICABLE

**4. REFERENCE TO A "SEQUENCE LISTING," A
TABLE, OR A COMPUTER PROGRAM LISTING
APPENDIX SUBMITTED ON A COMPACT
DISK**

The sequence information in the paper copy of the Sequence Listing filed herewith is identical to the sequence information in the only computer readable form which was filed on Apr. 29, 2011 in application Ser. No. 13/098,391 filed Apr. 29, 2011. A request for Transfer of a Computer Readable Form Under 37 C.F.R. § 1.821(e) accompanies this filing.

5. FIELD OF THE INVENTION

The present invention is directed to a Drug Compound and more particularly to Drug-Linker-Ligand Conjugates, Drug-Linker Compounds, and Drug-Ligand Conjugates, to compositions including the same, and to methods for using the same to treat cancer, an autoimmune disease or an infectious disease. The present invention is also directed to antibody-drug conjugates, to compositions including the same, and to methods for using the same to treat cancer, an autoimmune disease or an infectious disease. The invention also relates to methods of using antibody-drug conjugate

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compounds for in vitro, in situ, and in vivo diagnosis or treatment of mammalian cells, or associated pathological conditions.

6. BACKGROUND OF THE INVENTION

Improving the delivery of drugs and other agents to target cells, tissues and tumors to achieve maximal efficacy and minimal toxicity has been the focus of considerable research for many years. Though many attempts have been made to develop effective methods for importing biologically active molecules into cells, both in vivo and in vitro, none has proved to be entirely satisfactory. Optimizing the association of the drug with its intracellular target, while minimizing intercellular redistribution of the drug, e.g., to neighboring cells, is often difficult or inefficient.

Most agents currently administered to a patient parenterally are not targeted, resulting in systemic delivery of the agent to cells and tissues of the body where it is unnecessary, and often undesirable. This may result in adverse drug side effects, and often limits the dose of a drug (e.g., chemotherapeutic (anti-cancer), cytotoxic, enzyme inhibitor agents and antiviral or antimicrobial drugs) that can be administered. By comparison, although oral administration of drugs is considered to be a convenient and economical mode of administration, it shares the same concerns of non-specific toxicity to unaffected cells once the drug has been absorbed into the systemic circulation. Further complications involve problems with oral bioavailability and residence of drug in the gut leading to additional exposure of gut to the drug and hence risk of gut toxicities. Accordingly, a major goal has been to develop methods for specifically targeting agents to cells and tissues. The benefits of such treatment include avoiding the general physiological effects of inappropriate delivery of such agents to other cells and tissues, such as uninfected cells. Intracellular targeting may be achieved by methods, compounds and formulations which allow accumulation or retention of biologically active agents, i.e. active metabolites, inside cells.

Monoclonal antibody therapy has been established for the targeted treatment of patients with cancer, immunological and angiogenic disorders.

The use of antibody-drug conjugates for the local delivery of cytotoxic or cytostatic agents, e.g., drugs to kill or inhibit tumor cells in the treatment of cancer (Syrigos and Epenetos (1999) *Anticancer Research* 19:605-614; Niculescu-Duvaz and Springer (1997) *Adv. Drug. Del. Rev.* 26:151-172; U.S. Pat. No. 4,975,278) theoretically allows targeted delivery of the drug moiety to tumors, and intracellular accumulation therein, while systemic administration of these unconjugated drug agents may result in unacceptable levels of toxicity to normal cells as well as the tumor cells sought to be eliminated (Baldwin et al., 1986, *Lancet* pp. (Mar. 15, 1986): 603-05; Thorpe, 1985, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review," in *Monoclonal Antibodies '84: Biological And Clinical Applications*, A. Pinchera et al. (ed.s), pp. 475-506). Maximal efficacy with minimal toxicity is sought thereby. Both polyclonal antibodies and monoclonal antibodies have been reported as useful in these strategies (Rowland et al., 1986, *Cancer Immunol. Immunother.* 21:183-87). Drugs used in these methods include daunomycin, doxorubicin, methotrexate, and vindesine (Rowland et al., 1986, *supra*). Toxins used in antibody-toxin conjugates include bacterial toxins such as diphtheria toxin, plant toxins such as ricin, small molecule toxins such as geldanamycin (Kerr et al., 1997, *Bioconjugate Chem.* 8(6):781-784; Mandler et al. (2000) *Jour. of the Nat.*

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Cancer Inst. 92(19):1573-1581; Mandler et al. (2000) Bioorganic & Med. Chem. Letters 10:1025-1028; Mandler et al. (2002) Bioconjugate Chem. 13:786-791), maytansinoids (EP 1391213; Liu et al., (1996) Proc. Natl. Acad. Sci. USA 93:8618-8623), and calicheamicin (Lode et al. (1998) Cancer Res. 58:2928; Hinman et al. (1993) Cancer Res. 53:3336-3342). The toxins may affect their cytotoxic and cytostatic effects by mechanisms including tubulin binding, DNA binding, or topoisomerase inhibition (Meyer, D. L. and Senter, P. D. "Recent Advances in Antibody Drug Conjugates for Cancer Therapy" in Annual Reports in Medicinal Chemistry, Vol 38 (2003) Chapter 23, 229-237). Some cytotoxic drugs tend to be inactive or less active when conjugated to large antibodies or protein receptor ligands.

ZEVALIN® (ibritumomab tiuxetan, Biogen/Idex) is an antibody-radioisotope conjugate composed of a murine IgG1 kappa monoclonal antibody directed against the CD20 antigen found on the surface of normal and malignant B lymphocytes and ¹¹¹In or ⁹⁰Y radioisotope bound by a thiourea linker-chelator (Wiseman et al. (2000) Eur. Jour. Nucl. Med. 27(7):766-77; Wiseman et al. (2002) Blood 99(12):4336-42; Witzig et al. (2002) J. Clin. Oncol. 20(10):2453-63; Witzig et al. (2002) J. Clin. Oncol. 20(15):3262-69). Although ZEVALIN has activity against B-cell non-Hodgkin's Lymphoma (NHL), administration results in severe and prolonged cytopenias in most patients. MYLOTARG™ (gemtuzumab ozogamicin, Wyeth Pharmaceuticals), an antibody drug conjugate composed of a hu CD33 antibody linked to calicheamicin, was approved in 2000 for the treatment of acute myeloid leukemia by injection (Drugs of the Future (2000) 25(7):686; U.S. Pat. Nos. 4,970,198; 5,079,233; 5,585,089; 5,606,040; 5,693,762; 5,739,116; 5,767,285; 5,773,001). Cantuzumab mertansine (Immunogen, Inc.), an antibody drug conjugate composed of the huC242 antibody linked via the disulfide linker SPP to the maytansinoid drug moiety, DM1, is advancing into Phase II trials for the treatment of cancers that express CanAg, such as colon, pancreatic, gastric, and others. MLN-2704 (Millennium Pharm., BZL Biologics, Immunogen Inc.), an antibody drug conjugate composed of the anti-prostate specific membrane antigen (PSMA) monoclonal antibody linked to the maytansinoid drug moiety, DM1, is under development for the potential treatment of prostate tumors. The same maytansinoid drug moiety, DM1, was linked through a non-disulfide linker, SMCC, to a mouse murine monoclonal antibody, TA.1 (Chari et al. (1992) Cancer Research 52:127-131). This conjugate was reported to be 200-fold less potent than the corresponding disulfide linker conjugate. The SMCC linker was considered therein to be "noncleavable."

Several short peptidic compounds have been isolated from the marine mollusc *Dolabella auricularia* and found to have biological activity (Pettit et al. (1993) Tetrahedron 49:9151; Nakamura et al. (1995) Tetrahedron Letters 36:5059-5062; Sone et al. (1995) Jour. Org. Chem. 60:4474). Analogs of these compounds have also been prepared, and some were found to have biological activity (for a review, see Pettit et al. (1998) Anti-Cancer Drug Design 13:243-277). For example, auristatin E (U.S. Pat. No. 5,635,483) is a synthetic analogue of the marine natural product Dolastatin 10, an agent that inhibits tubulin polymerization by binding to the same domain on tubulin as the anticancer drug vincristine (G. R. Pettit, (1997) Prog. Chem. Org. Nat. Prod. 70:1-79). Dolastatin 10, auristatin PE, and auristatin E are linear peptides having four amino acids, three of which are unique to the dolastatin class of compounds, and a C-terminal amide.

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The auristatin peptides, auristatin E (AE) and monomethylauristatin (MMAE), synthetic analogs of dolastatin, were conjugated to: (i) chimeric monoclonal antibodies cBR96 (specific to Lewis Y on carcinomas); (ii) cAC10 which is specific to CD30 on hematological malignancies (Klussman, et al. (2004), Bioconjugate Chemistry 15(4):765-773; Doronina et al. (2003) Nature Biotechnology 21(7):778-784; "Monomethylvaline Compounds Capable of Conjugation to Ligands"; Francisco et al. (2003) Blood 102(4):1458-1465; U.S. Publication 2004/0018194; (iii) anti-CD20 antibodies such as RITUXAN® (WO 04/032828) for the treatment of CD20-expressing cancers and immune disorders; (iv) anti-EphB2 antibodies 2H9 and anti-IL-8 for treatment of colorectal cancer (Mao, et al. (2004) Cancer Research 64(3):781-788); (v) E-selectin antibody (Bhaskar et al. (2003) Cancer Res. 63:6387-6394); and (vi) other anti-CD30 antibodies (WO 03/043583).

Auristatin E conjugated to monoclonal antibodies are disclosed in Senter et al, Proceedings of the American Association for Cancer Research, Volume 45, Abstract Number 623, presented Mar. 28, 2004.

Despite in vitro data for compounds of the dolastatin class and its analogs, significant general toxicities at doses required for achieving a therapeutic effect compromise their efficacy in clinical studies. Accordingly, there is a clear need in the art for dolastatin/auristatin derivatives having significantly lower toxicity, yet useful therapeutic efficiency. These and other limitations and problems of the past are addressed by the present invention.

The ErbB family of receptor tyrosine kinases are important mediators of cell growth, differentiation and survival. The receptor family includes four distinct members including epidermal growth factor receptor (EGFR, ErbB1, HER1), HER2 (ErbB2 or p185^{neu}), HER3 (ErbB3) and HER4 (ErbB4 or tyro2). A panel of anti-ErbB2 antibodies has been characterized using the human breast tumor cell line SKBR3 (Hudziak et al., (1989) Mol. Cell. Biol. 9(3):1165-1172. Maximum inhibition was obtained with the antibody called 4D5 which inhibited cellular proliferation by 56%. Other antibodies in the panel reduced cellular proliferation to a lesser extent in this assay. The antibody 4D5 was further found to sensitize ErbB2-overexpressing breast tumor cell lines to the cytotoxic effects of TNF-α (U.S. Pat. No. 5,677,171). The anti-ErbB2 antibodies discussed in Hudziak et al. are further characterized in Fendly et al. (1990) Cancer Research 50:1550-1558; Kotts et al. (1990) In vitro 26(3):59A; Sarup et al. (1991) Growth Regulation 1:72-82; Shepard et al. J. (1991) Clin. Immunol. 11(3):117-127; Kumar et al. (1991) Mol. Cell. Biol. 11(2):979-986; Lewis et al. (1993) Cancer Immunol. Immunother. 37:255-263; Pietras et al. (1994) Oncogene 9:1829-1838; Vitetta et al. (1994) Cancer Research 54:5301-5309; Sliwkowski et al. (1994) J. Biol. Chem. 269(20):14661-14665; Scott et al. (1991) J. Biol. Chem. 266:14300-5; D'souza et al. Proc. Natl. Acad. Sci. (1994) 91:7202-7206; Lewis et al. (1996) Cancer Research 56:1457-1465; and Schaefer et al. (1997) Oncogene 15:1385-1394.

Other anti-ErbB2 antibodies with various properties have been described in Tagliabue et al. Int. J. Cancer 47:933-937 (1991); McKenzie et al. Oncogene 4:543-548 (1989); Maier et al. Cancer Res. 51:5361-5369 (1991); Bacus et al. Molecular Carcinogenesis 3:350-362 (1990); Stancovski et al. Proc. Natl. Acad. Sci. USA 88:8691-8695 (1991); Bacus et al. Cancer Research 52:2580-2589 (1992); Xu et al. Int. J. Cancer 53:401-408 (1993); WO94/00136; Kasprzyk et al. Cancer Research 52:2771-2776 (1992); Hancock et al. (1991) Cancer Res. 51:4575-4580; Shawver et al. (1994)

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Cancer Res. 54:1367-1373; Arteaga et al. (1994) Cancer Res. 54:3758-3765; Harwerth et al. (1992) J. Biol. Chem. 267:15160-15167; U.S. Pat. No. 5,783,186; and Klapper et al. (1997) Oncogene 14:2099-2109.

Homology screening has resulted in the identification of two other ErbB receptor family members; ErbB3 (U.S. Pat. Nos. 5,183,884; 5,480,968; Kra U. S. et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:9193-9197) and ErbB4 (EP 599274; Plowman et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:1746-1750; and Plowman et al. (1993) *Nature* 366:473-475). Both of these receptors display increased expression on at least some breast cancer cell lines. HERCEPTIN® (Trastuzumab) is a recombinant DNA-derived humanized monoclonal antibody that selectively binds with high affinity in a cell-based assay ($K_d=5$ nM) to the extracellular domain of the human epidermal growth factor receptor2 protein, HER2 (ErbB2) (U.S. Pat. Nos. 5,821,337; 6,054,297; 6,407,213; 6,639,055; Coussens L, et al. (1985) *Science* 230:1132-9; Slamon D J, et al. (1989) *Science* 244:707-12). Trastuzumab is an IgG1 kappa antibody that contains human framework regions with the complementarity-determining regions of a murine antibody (4D5) that binds to HER2. Trastuzumab binds to the HER2 antigen and thus, inhibits the growth of cancerous cells. Because Trastuzumab is a humanized antibody, it minimizes any HAMA response in patients. The humanized antibody against HER2 is produced by a mammalian cell (Chinese Hamster Ovary, CHO) suspension culture. The HER2 (or c-erbB2) proto-oncogene encodes a transmembrane receptor protein of 185 kDa, which is structurally related to the epidermal growth factor receptor. HER2 protein overexpression is observed in 25%-30% of primary breast cancers and can be determined using an immunohistochemistry based assessment of fixed tumor blocks (Press M F, et al. (1993) *Cancer Res* 53:4960-70. Trastuzumab has been shown, in both in vitro assays and in animals, to inhibit the proliferation of human tumor cells that overexpress HER2 (Hudziak R M, et al. (1989) *Mol Cell Biol* 9:1165-72; Lewis G D, et al. (1993) *Cancer Immunol Immunother*; 37:255-63; Baselga J, et al. (1998) *Cancer Res.* 58:2825-2831). Trastuzumab is a mediator of antibody-dependent cellular cytotoxicity, ADCC (Hotelling T E, et al. (1996) [abstract]. *Proc. Annual Meeting Am Assoc Cancer Res*; 37:471; Pegram M D, et al. (1997) [abstract]. *Proc Am Assoc Cancer Res*; 38:602). In vitro, Trastuzumab mediated ADCC has been shown to be preferentially exerted on HER2 overexpressing cancer cells compared with cancer cells that do not overexpress HER2. HERCEPTIN® as a single agent is indicated for the treatment of patients with metastatic breast cancer whose tumors overexpress the HER2 protein and who have received one or more chemotherapy regimens for their metastatic disease. HERCEPTIN® in combination

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with paclitaxel is indicated for treatment of patients with metastatic breast cancer whose tumors overexpress the HER2 protein and who have not received chemotherapy for their metastatic disease. HERCEPTIN® is clinically active in patients with ErbB2-overexpressing metastatic breast cancers that have received extensive prior anti-cancer therapy (Baselga et al, (1996) *J. Clin. Oncol.* 14:737-744).

The murine monoclonal anti-HER2 antibody inhibits the growth of breast cancer cell lines that overexpress HER2 at the 2+ and 3+(1-2×10⁶ HER2 receptors per cell) level, but has no activity on cells that express lower levels of HER2 (Lewis et al., (1993) *Cancer Immunol. Immunother.* 37:255-263). Based on this observation, antibody 4D5 was humanized (huMAb4D5-8, rhuMAb HER2, U.S. Pat. No. 5,821,337; Carter et al., (1992) *Proc. Natl. Acad. Sci. USA* 89:4285-4289) and tested in breast cancer patients whose tumors overexpress HER2 but who had progressed after conventional chemotherapy (Cobleigh et al., (1999) *J. Clin. Oncol.* 17: 2639-2648).

Although HERCEPTIN is a breakthrough in treating patients with ErbB2-overexpressing breast cancers that have received extensive prior anti-cancer therapy, some patients in this population fail to respond or respond only poorly to HERCEPTIN treatment.

Therefore, there is a significant clinical need for developing further HER2-directed cancer therapies for those patients with HER2-overexpressing tumors or other diseases associated with HER2 expression that do not respond, or respond poorly, to HERCEPTIN treatment.

The recitation of any reference in this application is not an admission that the reference is prior art to this application.

7. SUMMARY OF THE INVENTION

In one aspect, the present invention provides Drug-Linker-Ligand compounds having the Formula Ia:



or a pharmaceutically acceptable salt or solvate thereof wherein,

L- is a Ligand unit;

-A_x-W_w-Y_y- is a Linker unit (LU), wherein the Linker unit includes:

-A- is a Stretcher unit,

a is 0 or 1,

each -W- is independently an Amino Acid unit,

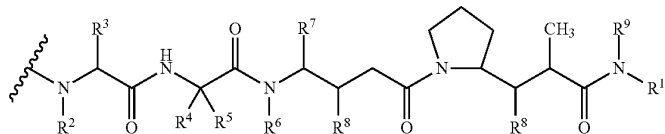
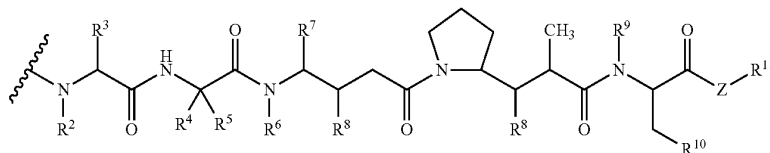
w is an integer ranging from 0 to 12,

-Y- is a Spacer unit, and

y is 0, 1 or 2;

p ranges from 1 to about 20; and

-D is a Drug unit having the Formulas D_E and D_F:

D_ED_F

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wherein, independently at each location:

R² is selected from H and C₁-C₈ alkyl;

R³ is selected from H, C₁-C₈ alkyl, C₃-C₈ carbocycle, aryl, C₁-C₈ alkyl-aryl, C₁-C₈ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₈ alkyl-(C₃-C₈ heterocycle);

R⁴ is selected from H, C₁-C₈ alkyl, C₃-C₈ carbocycle, aryl, C₁-C₈ alkyl-aryl, C₁-C₈ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₈ alkyl-(C₃-C₈ heterocycle);

R⁵ is selected from H and methyl;

or R⁴ and R⁵ jointly form a carbocyclic ring and have the formula —(CR^aR^b)_n— wherein R^a and R^b are independently selected from H, C₁-C₈ alkyl and C₃-C₈ carbocycle and n is selected from 2, 3, 4, 5 and 6;

R⁶ is selected from H and C₁-C₈ alkyl;

R⁷ is selected from H, C₁-C₈ alkyl, C₃-C₈ carbocycle, aryl, C₁-C₈ alkyl-aryl, C₁-C₈ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₈ alkyl-(C₃-C₈ heterocycle);

each R⁸ is independently selected from H, OH, C₁-C₈ alkyl, C₃-C₈ carbocycle and O—(C₁-C₈ alkyl);

R⁹ is selected from H and C₁-C₈ alkyl;

R¹⁰ is selected from aryl or C₃-C₈ heterocycle;

Z is O, S, NH, or NR¹², wherein R¹² is C₁-C₈ alkyl;

R¹¹ is selected from H, C₁-C₂₀ alkyl, aryl, C₃-C₈ heterocycle, —(R¹³O)_m—R¹⁴, or —(R¹³O)_m—CH(R¹⁵)₂;

m is an integer ranging from 1-1000;

R¹³ is C₂-C₈ alkyl;

R¹⁴ is H or C₁-C₈ alkyl;

each occurrence of R¹⁵ is independently H, COOH, —(CH₂)_n—N(R¹⁶)₂, —(CH₂)_n—SO₃H, or —(CH₂)_n—SO₃—C₁-C₈ alkyl;

each occurrence of R¹⁶ is independently H, C₁-C₈ alkyl, or —(CH₂)_n—COOH;

where; n is an integer ranging from 0 to 6; and

R¹⁸ is selected from —C(R⁸)₂—C(R⁸)₂—aryl, —C(R⁸)₂—C(R⁸)₂—(C₃-C₈ heterocycle), and —C(R⁸)₂—C(R⁸)₂—(C₃-C₈ carbocycle).

In another aspect, Drug Compounds having the Formula Ib are provided:

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alkyl-(C₃-C₈ heterocycle) wherein R⁵ is selected from —H and -methyl; or R⁴ and R⁵ jointly, have the formula —(CR^aR^b)_n— wherein R^a and R^b are independently selected from —H, —C₁-C₈ alkyl and —C₃-C₈ carbocycle and n is selected from 2, 3, 4, 5 and 6, and form a ring with the carbon atom to which they are attached;

R⁶ is selected from H and —C₁-C₈ alkyl;

R⁷ is selected from H, —C₁-C₈ alkyl, —C₃-C₈ carbocycle, aryl, —C₁-C₈ alkyl-aryl, —C₁-C₈ alkyl-(C₃-C₈ carbocycle), —C₃-C₈ heterocycle and —C₁-C₈ alkyl-(C₃-C₈ heterocycle);

each R⁸ is independently selected from H, —OH, —C₁-C₈ alkyl, —C₃-C₈ carbocycle and —O—(C₁-C₈ alkyl);

R⁹ is selected from H and —C₁-C₈ alkyl;

R¹⁰ is selected from aryl group or —C₃-C₈ heterocycle; Z is —O—, —S—, —NH—, or —NR¹²—, wherein R¹² is C₁-C₈ alkyl;

R¹¹ is selected from H, C₁-C₂₀ alkyl, aryl, —C₃-C₈ heterocycle, —(R¹³O)_m—R¹⁴, or —(R¹³O)_m—CH(R¹⁵)₂;

m is an integer ranging from 1-1000;

R¹³ is —C₂-C₈ alkyl; R¹⁴ is H or —C₁-C₈ alkyl;

each occurrence of R¹⁵ is independently H, —COOH, —(CH₂)_n—N(R¹⁶)₂, —(CH₂)_n—SO₃H, or —(CH₂)_n—SO₃—C₁-C₈ alkyl;

each occurrence of R¹⁶ is independently H, —C₁-C₈ alkyl, or —(CH₂)_n—COOH; and

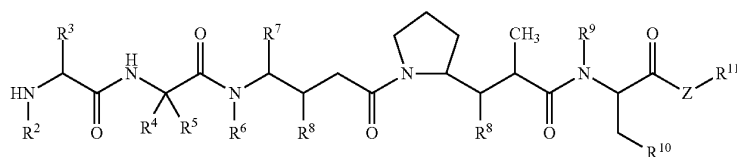
n is an integer ranging from 0 to 6.

The compounds of Formula (Ib) are useful for treating cancer, an autoimmune disease or an infectious disease in a patient or useful as an intermediate for the synthesis of a Drug-Linker, Drug-Linker-Ligand Conjugate, and Drug-Ligand Conjugate having a cleavable Drug unit.

In another aspect, compositions are provided including an effective amount of a Drug-Linker-Ligand Conjugate and a pharmaceutically acceptable carrier or vehicle.

In still another aspect, the invention provides pharmaceutical compositions comprising an effective amount of a Drug-Linker Compound and a pharmaceutically acceptable carrier or vehicle.

In still another aspect, the invention provides compositions comprising an effective amount of a Drug-Ligand Conjugate having a cleavable Drug unit from the Drug-Ligand Conjugate and a pharmaceutically acceptable carrier or vehicle.



Ib

or pharmaceutically acceptable salts or solvates thereof, wherein:

R² is selected from hydrogen and —C₁-C₈ alkyl;

R³ is selected from hydrogen, —C₁-C₈ alkyl, —C₃-C₈ carbocycle, aryl, —C₁-C₈ alkyl-aryl, —C₁-C₈ alkyl-(C₃-C₈ carbocycle), —C₃-C₈ heterocycle and —C₁-C₈ alkyl-(C₃-C₈ heterocycle);

R⁴ is selected from hydrogen, —C₁-C₈ alkyl, —C₃-C₈ carbocycle, —aryl, —C₁-C₈ alkyl-aryl, —C₁-C₈ alkyl-(C₃-C₈ carbocycle), —C₃-C₈ heterocycle and —C₁-C₈

In yet another aspect, the invention provides methods for killing or inhibiting the multiplication of a tumor cell or cancer cell including administering to a patient in need thereof an effective amount of a Drug-Linker Compound.

In another aspect, the invention provides methods for killing or inhibiting the multiplication of a tumor cell or cancer cell including administering to a patient in need thereof an effective amount of a Drug-Linker-Ligand Conjugate.

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In another aspect, the invention provides methods for preventing the multiplication of a tumor cell or cancer cell

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In another aspect, a Drug Compound is provided which can be used as an intermediate for the synthesis of a Drug-Linker Compound having a cleavable Drug unit from the Drug-Ligand

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Conjugate.

In another aspect, a Drug-Linker Compound is provided which can be used as an intermediate for the synthesis of a Drug-Linker-Ligand Conjugate.

In another aspect, compounds having Formula Ia' are 5 provided:



or a pharmaceutically acceptable salt or solvate thereof, wherein:

Ab includes an antibody including one which binds to 10 CD30, CD40, CD70, and Lewis Y antigen,

A is a Stretcher unit,

a is 0 or 1,

each W is independently an Amino Acid unit,

w is an integer ranging from 0 to 12,

Y is a Spacer unit, and

y is 0, 1 or 2,

p ranges from 1 to about 20, and

D is a Drug unit selected from Formulas D_E and D_F:

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R¹⁴ is H or C₁-C₈ alkyl;

each occurrence of R¹⁵ is independently H, COOH,

—(CH₂)_n—N(R¹⁶)₂, —(CH₂)_n—SO₃H, or

—(CH₂)_n—SO₃—C₁-C₈ alkyl;

each occurrence of R¹⁶ is independently H, C₁-C₈ alkyl,

or —(CH₂)_n—COOH;

R¹⁸ is selected from —C(R⁸)₂—C(R⁸)₂-aryl, —C(R⁸)₂—

C(R⁸)₂—(C₃-C₈ heterocycle), and —C(R⁸)₂—

C(R⁸)₂—(C₃-C₈ carbocycle); and

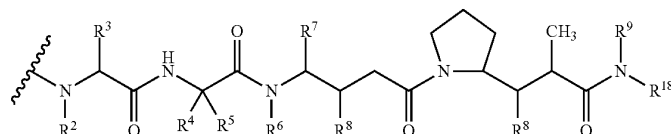
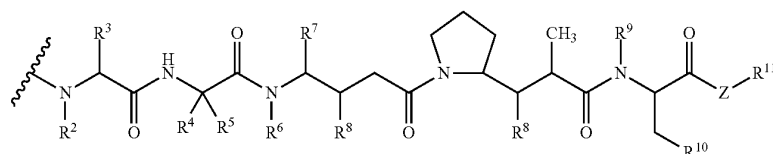
n is an integer ranging from 0 to 6.

In one embodiment, Ab is not an antibody which binds to an ErbB receptor or which binds to one or more of receptors (1)-(35):

(1) BMPR1B (bone morphogenetic protein receptor-type 1B, Genbank accession no. NM_001203);

(2) E16 (LAT1, SLC7A5, Genbank accession no. NM_003486);

(3) STEAP1 (six transmembrane epithelial antigen of prostate, Genbank accession no. NM_012449);

D_ED_F

wherein, independently at each location:

R² is selected from H and C₁-C₈ alkyl;

R³ is selected from H, C₁-C₈ alkyl, C₃-C₈ carbocycle, 40 aryl, C₁-C₈ alkyl-aryl, C₁-C₈ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₈ alkyl-(C₃-C₈ heterocycle);

R⁴ is selected from H, C₁-C₈ alkyl, C₃-C₈ carbocycle, 45 aryl, C₁-C₈ alkyl-aryl, C₁-C₈ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₈ alkyl-(C₃-C₈ heterocycle);

R⁵ is selected from H and methyl;

or R⁴ and R⁵ jointly form a carbocyclic ring and have the 50 formula —(C^aR^b)_n— wherein R^a and R^b are independently selected from H, C₁-C₈ alkyl and C₃-C₈ carbocycle and n is selected from 2, 3, 4, 5 and 6;

R⁶ is selected from H and C₁-C₈ alkyl;

R⁷ is selected from H, C₁-C₈ alkyl, C₃-C₈ carbocycle, 55 aryl, C₁-C₈ alkyl-aryl, C₁-C₈ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₈ alkyl-(C₃-C₈ heterocycle);

each R⁸ is independently selected from H, OH, C₁-C₈ 60 alkyl, C₃-C₈ carbocycle and O—(C₁-C₈ alkyl);

R⁹ is selected from H and C₁-C₈ alkyl;

R¹⁰ is selected from aryl or C₃-C₈ heterocycle;

Z is O, S, NH, or NR¹², wherein R¹² is C₁-C₈ alkyl;

R¹¹ is selected from H, C₁-C₂₀ alkyl, aryl, C₃-C₈ hetero- 65 cycle, —(R¹³O)_m—R¹⁴, or —(R¹³O)_m—CH(R¹⁵)₂;

m is an integer ranging from 1-1000;

R¹³ is C₂-C₈ alkyl;

(4) 0772P (CA125, MUC16, Genbank accession no. AF361486);

(5) MPF (MPF, MSLN, SMR, megakaryocyte potentiating factor, mesothelin, Genbank accession no. NM_005823);

(6) Napi3b (NAPI-3B, NPTIIB, SLC34A2, solute carrier family 34 (sodium phosphate), member 2, type II sodium-dependent phosphate transporter 3b, Genbank accession no. NM_006424);

(7) Sema 5b (F1110372, KIAA1445, Mm.42015, SEMA5B, SEMAG, Semaphorin 5b Hlog, sema domain, seven thrombospondin repeats (type 1 and type 1-like), transmembrane domain 1 and short cytoplasmic domain, (18semaphoring) 5B, Genbank accession no. AB040878);

(8) PSCA hlg (2700050C12Rik, C530008016Rik, RIKEN Cdna 2700050C12, RIKEN Cdna 2700050C12 gene, Genbank accession no. AY358628);

(9) ETBR (Endothelin type B receptor, Genbank accession no. AY275463);

(10) MSG783 (RNF124, hypothetical protein FLJ20315, Genbank accession no. NM_017763);

(11) STEAP2 (HGNC_8639, IPCA-1, PCANAP1, STAMP1, STEAP2, STMP, prostate cancer associated gene 1, prostate cancer associated protein 1, six transmembrane epithelial antigen of prostate 2, six transmembrane prostate protein, Genbank accession no. AF455138);

(12) TrpM4 (BR22450, F1120041, TRPM4, TRPM4B, transient receptor potential cation channel, subfamily M, member 4, Genbank accession no. NM_017636);

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- (13) CRIPTO (CR, CR1, CRGE, CRIPTO, TDGF1, teratocarcinoma-derived growth factor, Genbank accession no. NP_003203 or NM_003212);
- (14) CD21 (CR2 (Complement receptor 2) or c3DR (C3d/Epstein Barr virus receptor) or Hs.73792, Genbank accession no. M26004);
- (15) CD79b (Igb (immunoglobulin-associated beta), B29, Genbank accession no. NM_000626);
- (16) FcRH2 (IFGP4, IRTA4, SPAP1A (SH2 domain containing phosphatase anchor protein 1a), SPAP1B, SPAP1C, Genbank accession no. NM_030764);
- (17) HER2 (Genbank accession no. M11730);
- (18) NCA (Genbank accession no. M18728);
- (19) MDP (Genbank accession no. BC017023);
- (20) IL20R α (Genbank accession no. AF184971);
- (21) Brevican (Genbank accession no. AF229053);
- (22) Ephb2R (Genbank accession no. NM_004442);
- (23) ASLG659 (Genbank accession no. AX092328);
- (24) PSCA (Genbank accession no. AJ297436);
- (25) GEDA (Genbank accession no. AY260763);
- (26) BAFF-R (Genbank accession no. NP_443177.1);
- (27) CD22 (Genbank accession no. NP_001762.1);
- (28) CD79a (CD79A, CD79a, immunoglobulin-associated alpha, a B cell-specific protein that covalently interacts with Ig beta (CD79B) and forms a complex on the surface with Ig M molecules, transduces a signal involved in B-cell differentiation, Genbank accession No. NP_001774.1);
- (29) CXCR5 (Burkitt's lymphoma receptor 1, a G protein-coupled receptor that is activated by the CXCL13 chemokine, functions in lymphocyte migration and humoral defense, plays a role in HIV-2 infection and perhaps development of AIDS, lymphoma, myeloma, and leukemia, Genbank accession No. NP_001707.1);
- (30) HLA-DOB (Beta subunit of MHC class II molecule (Ia antigen) that binds peptides and presents them to CD4+T lymphocytes, Genbank accession No. NP_002111.1);
- (31) P2X5 (Purinergic receptor P2X ligand-gated ion channel 5, an ion channel gated by extracellular ATP, may be involved in synaptic transmission and neurogenesis, deficiency may contribute to the pathophysiology of idiopathic detrusor instability, Genbank accession No. NP_002552.2);
- (32) CD72 (B-cell differentiation antigen CD72, Lyb-2, Genbank accession No. NP_001773.1);
- (33) LY64 (Lymphocyte antigen 64 (RP105), type I membrane protein of the leucine rich repeat (LRR) family, regulates B-cell activation and apoptosis, loss of function is associated with increased disease activity in patients with systemic lupus erythematosus, Genbank accession No. NP_005573.1);
- (34) FCRH1 (Fc receptor-like protein 1, a putative receptor for the immunoglobulin Fc domain that contains C₂ type Ig-like and ITAM domains, may have a role in B-lymphocyte differentiation, Genbank accession No. NP_443170.1); or
- (35) IRTA2 (Immunoglobulin superfamily receptor translocation associated 2, a putative immunoreceptor with possible roles in B cell development and lymphomagenesis; deregulation of the gene by translocation occurs in some B cell malignancies, Genbank accession No. NP_112571.1).

In still another aspect, the invention provides pharmaceutical compositions comprising an effective amount of a Drug-Linker-Antibody Conjugate and a pharmaceutically acceptable carrier or vehicle.

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In still another aspect, the invention provides compositions comprising an effective amount of a Drug-Antibody Conjugate having a cleavable Drug unit (moiety) from the Drug-Antibody Conjugate and a pharmaceutically acceptable carrier or vehicle.

In another aspect, the invention provides methods for killing or inhibiting the multiplication of a tumor cell or cancer cell including administering to a patient in need thereof an effective amount of a Drug-Linker-Antibody Conjugate.

In another aspect, the invention provides methods for killing or inhibiting the multiplication of a tumor cell or cancer cell including administering to a patient in need thereof an effective amount of a Drug-Antibody Conjugate having a cleavable Drug unit from the Drug-Antibody Conjugate.

In yet another aspect, the invention provides methods for treating cancer including administering to a patient in need thereof an effective amount of a Drug-Linker-Antibody Conjugate.

In yet another aspect, the invention provides methods for treating cancer including administering to a patient in need thereof an effective amount of a Drug-Antibody Conjugate having a cleavable Drug unit from the Drug-Antibody Conjugate.

In another aspect, the invention provides methods for killing or inhibiting the replication of a cell that expresses an autoimmune antibody including administering to a patient in need thereof an effective amount of a Drug-Linker-Antibody Conjugate.

In another aspect, the invention provides methods for killing or inhibiting the replication of a cell that expresses an autoimmune antibody including administering to a patient in need thereof an effective amount of a Drug-Antibody Conjugate having a cleavable Drug unit from the Drug-Antibody Conjugate.

In yet another aspect, the invention provides methods for treating an autoimmune disease including administering to a patient in need thereof an effective amount of a Drug-Linker-Antibody Conjugate.

In yet another aspect, the invention provides methods for treating an autoimmune disease including administering to a patient in need thereof an effective amount of a Drug-Antibody Conjugate having a cleavable Drug unit from the Drug-Antibody Conjugate.

In still another aspect, the invention provides methods for treating an infectious disease including administering to a patient in need thereof an effective amount of a Drug-Linker-Antibody Conjugate.

In still another aspect, the invention provides methods for treating an infectious disease including administering to a patient in need thereof an effective amount of a Drug-Antibody Conjugate having a cleavable Drug unit from the Drug-Antibody Conjugate.

In another aspect, the invention provides methods for preventing the multiplication of a tumor cell or cancer cell including administering to a patient in need thereof an effective amount of a Drug-Linker-Antibody Conjugate.

In another aspect, the invention provides methods for preventing the multiplication of a tumor cell or cancer cell including administering to a patient in need thereof an effective amount of a Drug-Antibody Conjugate having a cleavable Drug unit from the Drug-Antibody Conjugate.

In yet another aspect, the invention provides methods for preventing cancer including administering to a patient in need thereof an effective amount of a Drug-Linker-Antibody Conjugate.

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In yet another aspect, the invention provides methods for preventing cancer including administering to a patient in need thereof an effective amount of a Drug-Antibody Conjugate having a cleavable Drug unit from the Drug-Antibody Conjugate.

In another aspect, the invention provides methods for preventing the multiplication of a cell that expresses an autoimmune antibody including administering to a patient in need thereof an effective amount of a Drug-Linker-Antibody Conjugate.

In another aspect, the invention provides methods for preventing the multiplication of a cell that expresses an autoimmune antibody including administering to a patient in need thereof an effective amount of a Drug-Antibody Conjugate having a cleavable Drug unit from the Drug-Antibody Conjugate.

In yet another aspect, the invention provides methods for preventing an autoimmune disease including administering to a patient in need thereof an effective amount of a Drug-Linker-Antibody Conjugate.

In yet another aspect, the invention provides methods for preventing an autoimmune disease including administering to a patient in need thereof an effective amount of a Drug-Antibody Conjugate having a cleavable Drug unit from the Drug-Antibody Conjugate.

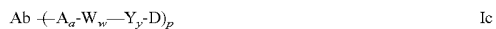
In still another aspect, the invention provides methods for preventing an infectious disease including administering to a patient in need thereof an effective amount of a Drug-Linker-Antibody Conjugate.

In still another aspect, the invention provides methods for preventing an infectious disease including administering to a patient in need thereof an effective amount of a Drug-Antibody Conjugate having a cleavable Drug unit from the Drug-Antibody Conjugate.

In another aspect, a Drug Compound is provided which can be used as an intermediate for the synthesis of a Drug-Linker Compound having a cleavable Drug unit from the Drug-Antibody Conjugate.

In another aspect, a Drug-Linker Compound is provided which can be used as an intermediate for the synthesis of a Drug-Linker-Antibody Conjugate.

In one aspect, the present invention provides Drug-Linker-Antibody Conjugates (also referred to as antibody-drug conjugates) having Formula Ic:



or a pharmaceutically acceptable salt or solvate thereof, wherein:

Ab is an antibody which binds to one or more of the antigens (1)-(35):

- (1) BMPR1B (bone morphogenetic protein receptor-type IB, Genbank accession no. NM_001203);
- (2) E16 (LAT1, SLC7A5, Genbank accession no. NM_003486);
- (3) STEAP1 (six transmembrane epithelial antigen of prostate, Genbank accession no. NM_012449);
- (4) 0772P (CA125, MUC16, Genbank accession no. AF361486);
- (5) MPF (MPF, MSLN, SMR, megakaryocyte potentiating factor, mesothelin, Genbank accession no. NM_005823);
- (6) Napi3b (NAPI-3B, NPTIIB, SLC34A2, solute carrier family 34 (sodium phosphate), member 2, type II sodium-dependent phosphate transporter 3b, Genbank accession no. NM_006424);
- (7) Sema 5b (FLJ10372, KIAA1445, Mm.42015, SEMA5B, SEMAG, Semaphorin 5b Hlog, sema

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domain, seven thrombospondin repeats (type 1 and type 1-like), transmembrane domain I and short cytoplasmic domain, (24emaphoring) 5B, Genbank accession no. AB040878);

- (8) PSCA hlg (2700050C12Rik, C₅₃₀₀₀₈O16Rik, RIKEN Cdna 2700050C12, RIKEN Cdna 2700050C12 gene, Genbank accession no. AY358628);
- (9) ETBR (Endothelin type B receptor, Genbank accession no. AY275463);
- (10) MSG783 (RNF124, hypothetical protein FLJ20315, Genbank accession no. NM_017763);
- (11) STEAP2 (HGNC_8639, IPCA-1, PCANAP1, STAMP1, STEAP2, STMP, prostate cancer associated gene 1, prostate cancer associated protein 1, six transmembrane epithelial antigen of prostate 2, six transmembrane prostate protein, Genbank accession no. AF455138);
- (12) TrpM4 (BR22450, F1120041, TRPM4, TRPM4B, transient receptor potential cation channel, subfamily M, member 4, Genbank accession no. NM_017636);
- (13) CRIPTO (CR, CR1, CRGF, CRIPTO, TDGF1, teratocarcinoma-derived growth factor, Genbank accession no. NP_003203 or NM_003212);
- (14) CD21 (CR2 (Complement receptor 2) or C3DR (C3d/Epstein Barr virus receptor) or Hs.73792, Genbank accession no. M26004);
- (15) CD79b (Igb (immunoglobulin-associated beta), B29, Genbank accession no. NM_000626);
- (16) FcRH2 (IFGP4, IRTA4, SPAP1A (SH2 domain containing phosphatase anchor protein 1a), SPAP1B, SPAP1C, Genbank accession no. NM_030764);
- (17) HER2 (Genbank accession no. M11730);
- (18) NCA (Genbank accession no. M18728);
- (19) MDP (Genbank accession no. BC017023);
- (20) IL20Rα (Genbank accession no. AF184971);
- (21) Brevican (Genbank accession no. AF229053);
- (22) Ephb2R (Genbank accession no. NM_004442);
- (23) ASLG659 (Genbank accession no. AX092328);
- (24) PSCA (Genbank accession no. AJ297436);
- (25) GEDA (Genbank accession no. AY260763);
- (26) BAFF-R (Genbank accession no. NP_443177.1);
- (27) CD22 (Genbank accession no. NP_001762.1);
- (28) CD79a (CD79A, CD79a, immunoglobulin-associated alpha, a B cell-specific protein that covalently interacts with Ig beta (CD79B) and forms a complex on the surface with Ig M molecules, transduces a signal involved in B-cell differentiation, Genbank accession No. NP_001774.1);
- (29) CXCR5 (Burkitt's lymphoma receptor 1, a G protein-coupled receptor that is activated by the CXCL13 chemokine, functions in lymphocyte migration and humoral defense, plays a role in HIV-2 infection and perhaps development of AIDS, lymphoma, myeloma, and leukemia, Genbank accession No. NP_001707.1);
- (30) HLA-DOB (Beta subunit of MHC class II molecule (Ia antigen) that binds peptides and presents them to CD4+T lymphocytes, Genbank accession No. NP_002111.1);
- (31) P2X5 (Purineric receptor P2X ligand-gated ion channel 5, an ion channel gated by extracellular ATP, may be involved in synaptic transmission and neurogenesis, deficiency may contribute to the pathophysiology of idiopathic detrusor instability, Genbank accession No. NP_002552.2);
- (32) CD72 (B-cell differentiation antigen CD72, Lyb-2, Genbank accession No. NP_001773.1);

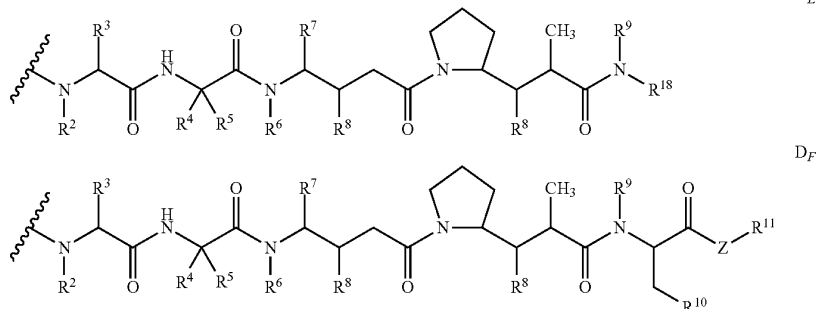
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- (33) LY64 (Lymphocyte antigen 64 (RP105), type I membrane protein of the leucine rich repeat (LRR) family, regulates B-cell activation and apoptosis, loss of function is associated with increased disease activity in patients with systemic lupus erythematosus, Genbank accession No. NP_005573.1);
- (34) FCRH1 (Fc receptor-like protein 1, a putative receptor for the immunoglobulin Fc domain that contains C₂ type Ig-like and ITAM domains, may have a role in B-lymphocyte differentiation, Genbank accession No. NP_443170.1); or
- (35) IRTA2 (Immunoglobulin superfamily receptor translocation associated 2, a putative immunoreceptor with possible roles in B cell development and lymphomagenesis; deregulation of the gene by translocation occurs in some B cell malignancies, Genbank accession No. NP_112571.1);
- A is a Stretcher unit,
a is 0 or 1,
each W is independently an Amino Acid unit,
w is an integer ranging from 0 to 12,
Y is a Spacer unit, and
y is 0, 1 or 2,
p ranges from 1 to about 20, and
D is a Drug moiety selected from Formulas D_E and D_F:

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- R⁹ is selected from H and C₁-C₈ alkyl;
R₁₀ is selected from aryl or C₃-C₈ heterocycle;
Z is O, S, NH, or NR¹², wherein R¹² is C₁-C₈ alkyl;
R¹¹ is selected from H, C₁-C₂₀ alkyl, aryl, C₃-C₈ heterocycle, $-(R^{13}O)_m-R^{14}$, or $-(R^{13}O)_m-CH(R^{15})_2$;
m is an integer ranging from 1-1000;
R¹³ is C₂-C₈ alkyl;
R¹⁴ is H or C₁-C₈ alkyl;
each occurrence of R¹⁵ is independently H, COOH, $-(CH_2)_n-N(R^{16})_2$, $-(CH_2)_n-SO_3H$, or $-(CH_2)_n-SO_3-C_1-C_8$ alkyl;
each occurrence of R¹⁶ is independently H, C₁-C₈ alkyl, or $-(CH_2)_n-COOH$;
R¹⁸ is selected from $-C(R^8)_2-C(R^8)_2$ -aryl, $-C(R^8)_2-C(R^8)_2-(C_3-C_8$ heterocycle), and $-C(R^8)_2-C(R^8)_2-(C_3-C_8$ carbocycle); and
n is an integer ranging from 0 to 6.
- In another aspect, the antibody of the antibody-drug conjugate (ADC) of the invention specifically binds to a receptor encoded by an ErbB2 gene.
- In another aspect, the antibody of the antibody-drug conjugate is a humanized antibody selected from huMAb4D5-1, huMAb4D5-2, huMAb4D5-3, huMAb4D5-4, huMAb4D5-5, huMAb4D5-6, huMAb4D5-7 and huMAb4D5-8 (Trastuzumab).



wherein the wavy line of D_E and D_F indicates the covalent attachment site to A, W, or Y, and independently at each location:

- R² is selected from H and C₁-C₈ alkyl;
R³ is selected from H, C₁-C₈ alkyl, C₃-C₈ carbocycle, aryl, C₁-C₈ alkyl-aryl, C₁-C₈ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₈ alkyl-(C₃-C₈ heterocycle);
R⁴ is selected from H, C₁-C₈ alkyl, C₃-C₈ carbocycle, aryl, C₁-C₈ alkyl-aryl, C₁-C₈ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₈ alkyl-(C₃-C₈ heterocycle);
R⁵ is selected from H and methyl;
or R⁴ and R⁵ jointly form a carbocyclic ring and have the formula $-(CR^aR^b)_n-$ wherein R^a and R^b are independently selected from H, C₁-C₈ alkyl and C₃-C₈ carbocycle and n is selected from 2, 3, 4, 5 and 6;
R⁶ is selected from H and C₁-C₈ alkyl;
R⁷ is selected from H, C₁-C₈ alkyl, C₃-C₈ carbocycle, aryl, C₁-C₈ alkyl-aryl, C₁-C₈ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₈ alkyl-(C₃-C₈ heterocycle);
each R⁸ is independently selected from H, OH, C₁-C₈ alkyl, C₃-C₈ carbocycle and O-(C₁-C₈ alkyl);

In another aspect, the invention includes an article of manufacture comprising an antibody-drug conjugate compound of the invention; a container; and a package insert or label indicating that the compound can be used to treat cancer characterized by the overexpression of an ErbB2 receptor.

In another aspect, the invention includes a method for the treatment of cancer in a mammal, wherein the cancer is characterized by the overexpression of an ErbB2 receptor and does not respond, or responds poorly, to treatment with an anti-ErbB2 antibody, comprising administering to the mammal a therapeutically effective amount of an antibody-drug conjugate compound of the invention.

In another aspect, a substantial amount of the drug moiety is not cleaved from the antibody until the antibody-drug conjugate compound enters a cell with a cell-surface receptor specific for the antibody of the antibody-drug conjugate, and the drug moiety is cleaved from the antibody when the antibody-drug conjugate does enter the cell.

In another aspect, the bioavailability of the antibody-drug conjugate compound or an intracellular metabolite of the compound in a mammal is improved when compared to a drug compound comprising the drug moiety of the antibody-drug conjugate compound, or when compared to an analog of the compound not having the drug moiety.

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In another aspect, the drug moiety is intracellularly cleaved in a mammal from the antibody of the compound, or an intracellular metabolite of the compound.

In another aspect, the invention includes a pharmaceutical composition comprising an effective amount of the antibody-drug conjugate compound of the invention, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable diluent, carrier or excipient. The composition may further comprise a therapeutically effective amount of chemotherapeutic agent such as a tubulin-forming inhibitor, a topoisomerase inhibitor, and a DNA binder.

In another aspect, the invention includes a method for killing or inhibiting the proliferation of tumor cells or cancer cells comprising treating tumor cells or cancer cells with an amount of the antibody-drug conjugate compound of the invention, or a pharmaceutically acceptable salt or solvate thereof, being effective to kill or inhibit the proliferation of the tumor cells or cancer cells.

In another aspect, the invention includes a method of inhibiting cellular proliferation comprising exposing mammalian cells in a cell culture medium to an antibody drug conjugate compound of the invention, wherein the antibody drug conjugate compound enters the cells and the drug is cleaved from the remainder of the antibody drug conjugate compound; whereby proliferation of the cells is inhibited.

In another aspect, the invention includes a method of treating cancer comprising administering to a patient a formulation of an antibody-drug conjugate compound of the invention and a pharmaceutically acceptable diluent, carrier or excipient.

In another aspect, the invention includes an assay for detecting cancer cells comprising:

- (a) exposing cells to an antibody-drug conjugate compound of the invention; and
- (b) determining the extent of binding of the antibody-drug conjugate compound to the cells.

The invention will best be understood by reference to the following detailed description of the exemplary embodiments, taken in conjunction with the accompanying drawings, figures, and schemes. The discussion below is descriptive, illustrative and exemplary and is not to be taken as limiting the scope defined by any appended claims.

8. BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows an in vivo, single dose, efficacy assay of cAC10-mcMMAF in subcutaneous Karpas-299 ALCL xenografts.

FIG. 2 shows an in vivo, single dose, efficacy assay of cAC10-mcMMAF in subcutaneous L540cy. For this study there were 4 mice in the untreated group and 10 in each of the treatment groups.

FIGS. 3a and 3b show in vivo efficacy of cBR96-mcMMAF in subcutaneous L2987. The filled triangles in FIG. 3a and arrows in FIG. 3b indicate the days of therapy.

FIGS. 4a and 4b show in vitro activity of cAC10-antibody-drug conjugates against CD30⁺ cell lines.

FIGS. 5a and 5b show in vitro activity of cBR96-antibody-drug conjugates against Le^y cell lines.

FIGS. 6a and 6b show in vitro activity of c1F6-antibody-drug conjugates against CD70⁺ renal cell carcinoma cell lines.

FIG. 7 shows an in vitro, cell proliferation assay with SK-BR-3 cells treated with antibody drug conjugates (ADC): -●- Trastuzumab-MC-vc-PAB-MMAF, 3.8 MMAF/Ab, -○- Trastuzumab-MC-MMAF, 4.1 MMAF/Ab, and -Δ- Trastuzumab-MC-MMAF, 4.8 MMAF/Ab, mea-

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sured in Relative Fluorescence Units (RLU) versus μg/ml concentration of ADC. H=Trastuzumab where H is linked via a cysteine [cys].

FIG. 8 shows an in vitro, cell proliferation assay with BT-474 cells treated with ADC: -●- Trastuzumab-MC-vc-PAB-MMAF, 3.8 MMAF/Ab, -○- Trastuzumab-MC-MMAF, 4.1 MMAF/Ab, and -Δ- Trastuzumab-MC-MMAF, 4.8 MMAF/Ab.

FIG. 9 shows an in vitro, cell proliferation assay with MCF-7 cells treated with ADC: -●- Trastuzumab-MC-vc-PAB-MMAF, 3.8 MMAF/Ab, -○- Trastuzumab-MC-(N-Me)vc-PAB-MMAF, 3.9 MMAF/Ab, and -Δ- Trastuzumab-MC-MMAF, 4.1 MMAF/Ab.

FIG. 10 shows an in vitro, cell proliferation assay with MDA-MB-468 cells treated with ADC: -●- Trastuzumab-MC-vc-PAB-MMAE, 4.1 MMAE/Ab, -○- Trastuzumab-MC-vc-PAB-MMAE, 3.3 MMAE/Ab, and -Δ- Trastuzumab-MC-vc-PAB-MMAE, 3.7 MMAE/Ab.

FIG. 11 shows a plasma concentration clearance study after administration of H-MC-vc-PAB-MMAF-TEG and H-MC-vc-PAB-MMAF to Sprague-Dawley rats: The administered dose was 2 mg of ADC per kg of rat. Concentrations of total antibody and ADC were measured over time. (H=Trastuzumab).

FIG. 12 shows a plasma concentration clearance study after administration of H-MC-vc-MMAE to Cynomolgus monkeys at different doses: 0.5, 1.5, 2.5, and 3.0 mg/kg administered at day 1 and day 21. Concentrations of total antibody and ADC were measured over time. (H=Trastuzumab).

FIG. 13 shows the mean tumor volume change over time in athymic nude mice with MMTV-HER2 Fo5 Mammary tumor allografts dosed on Day 0 with: Vehicle, Trastuzumab-MC-vc-PAB-MMAE (1250 μg/m²) and Trastuzumab-MC-vc-PAB-MMAF (555 μg/m²). (H=Trastuzumab).

FIG. 14 shows the mean tumor volume change over time in athymic nude mice with MMTV-HER2 Fo5 Mammary tumor allografts dosed on Day 0 with 10 mg/kg (660 μg/m²) of Trastuzumab-MC-MMAE and 1250 μg/m² Trastuzumab-MC-vc-PAB-MMAE.

FIG. 15 shows the mean tumor volume change over time in athymic nude mice with MMTV-HER2 Fo5 Mammary tumor allografts dosed on Day 0 with Vehicle and 650 μg/m² trastuzumab-MC-MMAF.

FIG. 16 shows the mean tumor volume change over time in athymic nude mice with MMTV-HER2 Fo5 Mammary tumor allografts dosed on Day 0 with Vehicle and 350 μg/m² of four trastuzumab-MC-MMAF conjugates where the MMAF/trastuzumab (H) ratio is 2, 4, 5.9 and 6.

FIG. 17 shows the Group mean change, with error bars, in animal (rat) body weights (Mean±SD) after administration of Vehicle, trastuzumab-MC-val-cit-MMAF, trastuzumab-MC(Me)-val-cit-PAB-MMAF, trastuzumab-MC-MMAF and trastuzumab-MC-val-cit-PAB-MMAF.

FIG. 18 shows the Group mean change in animal (rat) body weights (Mean SD) after administration of 9.94 mg/kg H-MC-vc-MMAF, 24.90 mg/kg H-MC-vc-MMAF, 10.69 mg/kg H-MC(Me)-vc-PAB-MMAF, 26.78 mg/kg H-MC(Me)-vc-PAB-MMAF, 10.17 mg/kg H-MC-MMAF, 25.50 mg/kg H-MC-MMAF, and 21.85 mg/kg H-MC-vc-PAB-MMAF. H=trastuzumab. The MC linker is attached via a cysteine of trastuzumab for each conjugate.

FIG. 19 shows the Group mean change, with error bars, in Sprague Dawley rat body weights (Mean±SD) after administration of trastuzumab (H)-MC-MMAF at doses of

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2105, 3158, and 4210 $\mu\text{g}/\text{m}^2$. The MC linker is attached via a cysteine of trastuzumab for each conjugate.

FIG. 20 shows examples of compounds with a non self-immolative Spacer unit.

FIG. 21 shows a scheme of a possible mechanism of Drug release from a PAB group which is attached directly to -D via a carbamate or carbonate group.

FIG. 22 shows a scheme of a possible mechanism of Drug release from a PAB group which is attached directly to -D via an ether or amine linkage.

FIG. 23 shows an example of a branched spacer unit, bis(hydroxymethyl)styrene (BHMS) unit, which can be used to incorporate and release multiple drug.

FIG. 24 shows a scheme of the CellTiter-Glo® Assay.

FIG. 25 shows the synthesis of an N-terminal tripeptide unit F which is a useful intermediate for the synthesis of the drug compounds of Formula Ib.

FIG. 26 shows the synthesis of an N-terminal tripeptide unit F which is a useful intermediate for the synthesis of the drug compounds of Formula Ib.

FIG. 27 shows the synthesis of an N-terminal tripeptide unit F which is a useful intermediate for the synthesis of the drug compounds of Formula Ib.

FIG. 28 shows the synthesis of useful linkers.

FIG. 29 shows the synthesis of useful linkers.

FIG. 30 shows a general synthesis of an illustrative Linker unit containing a maleimide Stretcher group and optionally a p-aminobenzyl ether self-immolative Spacer.

FIG. 31 shows the synthesis of a val-cit dipeptide Linker having a maleimide Stretcher and optionally a p-aminobenzyl self-immolative Spacer.

FIG. 32 shows the synthesis of a phe-lys(Mtr) dipeptide Linker unit having a maleimide Stretcher unit and a p-aminobenzyl self-immolative Spacer unit.

FIG. 33 shows the synthesis of a Drug-Linker Compound that contains an amide or carbamate group, linking the Drug unit to the Linker unit.

FIG. 34 shows illustrative methods useful for linking a Drug to a Ligand to form a Drug-Linker Compound.

FIG. 35 shows the synthesis of a val-cit dipeptide linker having a maleimide Stretcher unit and a bis(4-hydroxymethyl)styrene (BHMS) unit.

FIG. 36 shows methodology useful for making Drug-Linker-Ligand conjugates having about 2 to about 4 drugs per antibody.

FIG. 37 shows the synthesis of MC-MMAF via t-butyl ester.

FIG. 38 shows the synthesis of MC-MMAF (11) via dimethoxybenzyl ester.

FIG. 39 shows the synthesis of analog of mc-MMAF.

9. DETAILED DESCRIPTION OF THE EXEMPLARY EMBODIMENTS

9.1 Definitions and Abbreviations

Unless stated otherwise, the following terms and phrases as used herein are intended to have the following meanings:

When trade names are used herein, applicants intend to independently include the trade name product formulation, the generic drug, and the active pharmaceutical ingredient(s) of the trade name product.

The term “antibody” herein is used in the broadest sense and specifically covers intact monoclonal antibodies, polyclonal antibodies, multi specific antibodies (e.g., bispecific antibodies) formed from at least two intact antibodies, and antibody fragments, so long as they exhibit the desired

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biological activity. An antibody is a protein generated by the immune system that is capable of recognizing and binding to a specific antigen. Described in terms of its structure, an antibody typically has a Y-shaped protein consisting of four amino acid chains, two heavy and two light. Each antibody has primarily two regions: a variable region and a constant region. The variable region, located on the ends of the arms of the Y, binds to and interacts with the target antigen. This variable region includes a complementary determining region (CDR) that recognizes and binds to a specific binding site on a particular antigen. The constant region, located on the tail of the Y, is recognized by and interacts with the immune system (Janeway, C., Travers, P., Walport, M., Shlomchik (2001) *Immuno Biology*, 5th Ed., Garland Publishing, New York). A target antigen generally has numerous binding sites, also called epitopes, recognized by CDRs on multiple antibodies. Each antibody that specifically binds to a different epitope has a different structure. Thus, one antigen may have more than one corresponding antibody.

The term “antibody” as used herein, also refers to a full-length immunoglobulin molecule or an immunologically active portion of a full-length immunoglobulin molecule, i.e., a molecule that contains an antigen binding site that immunospecifically binds an antigen of a target of interest or part thereof, such targets including but not limited to, cancer cell or cells that produce autoimmune antibodies associated with an autoimmune disease. The immunoglobulin disclosed herein can be of any type (e.g., IgG, IgE, IgM, IgD, and IgA), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule. The immunoglobulins can be derived from any species. In one aspect, however, the immunoglobulin is of human, murine, or rabbit origin. In another aspect, the antibodies are polyclonal, monoclonal, bispecific, human, humanized or chimeric antibodies, single chain antibodies, Fv, Fab fragments, F(ab') fragments, F(ab')₂ fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies, CDR's, and epitope-binding fragments of any of the above which immunospecifically bind to cancer cell antigens, viral antigens or microbial antigens.

The term “monoclonal antibody” as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to polyclonal antibody preparations which include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they may be synthesized uncontaminated by other antibodies. The modifier “monoclonal” indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler et al. (1975) *Nature* 256:495, or may be made by recombinant DNA methods (see, U.S. Pat. No. 4,816,567). The “monoclonal antibodies” may also be isolated from phage antibody libraries using the techniques described in Clackson et al. (1991) *Nature*, 352:624-628 and Marks et al. (1991) *J. Mol. Biol.*, 222:581-597, for example.

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The monoclonal antibodies herein specifically include "chimeric" antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Pat. No. 4,816,567; and Morrison et al. (1984) *Proc. Natl. Acad. Sci. USA*, 81:6851-6855).

Various methods have been employed to produce monoclonal antibodies (MAbs). Hybridoma technology, which refers to a cloned cell line that produces a single type of antibody, uses the cells of various species, including mice (murine), hamsters, rats, and humans. Another method to prepare MAbs uses genetic engineering including recombinant DNA techniques. Monoclonal antibodies made from these techniques include, among others, chimeric antibodies and humanized antibodies. A chimeric antibody combines DNA encoding regions from more than one type of species. For example, a chimeric antibody may derive the variable region from a mouse and the constant region from a human. A humanized antibody comes predominantly from a human, even though it contains nonhuman portions. Like a chimeric antibody, a humanized antibody may contain a completely human constant region. But unlike a chimeric antibody, the variable region may be partially derived from a human. The nonhuman, synthetic portions of a humanized antibody often come from CDRs in murine antibodies. In any event, these regions are crucial to allow the antibody to recognize and bind to a specific antigen.

As noted, murine antibodies can be used. While useful for diagnostics and short-term therapies, murine antibodies cannot be administered to people long-term without increasing the risk of a deleterious immunogenic response. This response, called Human Anti-Mouse Antibody (HAMA), occurs when a human immune system recognizes the murine antibody as foreign and attacks it. A HAMA response can cause toxic shock or even death.

Chimeric and humanized antibodies reduce the likelihood of a HAMA response by minimizing the nonhuman portions of administered antibodies. Furthermore, chimeric and humanized antibodies have the additional benefit of activating secondary human immune responses, such as antibody dependent cellular cytotoxicity.

"Antibody fragments" comprise a portion of an intact antibody, preferably comprising the antigen-binding or variable region thereof. Examples of antibody fragments include Fab, Fab', F(ab')₂, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multi specific antibodies formed from antibody fragment(s).

An "intact" antibody is one which comprises an antigen-binding variable region as well as a light chain constant domain (CL) and heavy chain constant domains, CH₁, CH₂ and CH₃. The constant domains may be native sequence constant domains (e.g., human native sequence constant domains) or amino acid sequence variant thereof.

The intact antibody may have one or more "effector functions" which refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody. Examples of antibody effector functions include C1q binding; complement dependent cytotoxicity; Fc receptor binding; antibody-

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dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (e.g., B cell receptor; BCR), etc.

Depending on the amino acid sequence of the constant domain of their heavy chains, intact antibodies can be assigned to different "classes." There are five major classes of intact antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into "subclasses" (isotypes), e.g., IgG1, IgG2, IgG3, IgG4, IgA, and IgA2. The heavy-chain constant domains that correspond to the different classes of antibodies are called α , δ , ϵ , γ , and μ , respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

The expressions "ErbB2" and "HER2" are used interchangeably herein and refer to human HER2 protein described, for example, in Semba et al., *Proc. Natl. Acad. Sci. USA*, 82:6497-6501 (1985) and Yamamoto et al., (1986) *Nature*, 319:230-234 (Genebank accession number X03363). The term "erbB2" refers to the gene encoding human ErbB2 and "neu" refers to the gene encoding rat p185neu. Preferred ErbB2 is native sequence human ErbB2.

Antibodies to ErbB receptors are available commercially from a number of sources, including, for example, Santa Cruz Biotechnology, Inc., California, USA.

By "ErbB ligand" is meant a polypeptide which binds to and/or activates an ErbB receptor. The ErbB ligand may be a native sequence human ErbB ligand such as epidermal growth factor (EGF) (Savage et al. (1972) *J. Biol. Chem.*, 247:7612-7621); transforming growth factor alpha (TGF- α) (Marquardt et al. (1984) *Science* 223:1079-1082); amphiregulin also known as schwannoma or keratinocyte autocrine growth factor (Shoyab et al. (1989) *Science* 243:1074-1076; Kimura et al., *Nature*, 348:257-260 (1990); and Cook et al., *Mol. Cell. Biol.*, 11:2547-2557 (1991)); betacellulin (Shing et al., *Science*, 259:1604-1607 (1993); and Sasada et al., *Biochem. Biophys. Res. Commun.*, 190:1173 (1993)); heparin-binding epidermal growth factor (HB-EGF) (Higashiyama et al., *Science*, 251:936-939 (1991)); epiregulin (Toyoda et al., *J. Biol. Chem.*, 270:7495-7500 (1995); and Komurasaki et al., *Oncogene*, 15:2841-2848 (1997)); a heregulin (see below); neuregulin-2 (NRG-2) (Carraway et al., *Nature*, 387:512-516 (1997)); neuregulin-3 (NRG-3) (Zhang et al., *Proc. Natl. Acad. Sci.*, 94:9562-9567 (1997)); neuregulin-4 (NRG-4) (Harari et al., *Oncogene*, 18:2681-89 (1999)) or cripto (CR-1) (Kannan et al., *J. Biol. Chem.*, 272(6):3330-3335 (1997)). ErbB ligands which bind EGFR include EGF, TGF- α , amphiregulin, betacellulin, HB-EGF and epiregulin. ErbB ligands which bind ErbB3 include heregulins. ErbB ligands capable of binding ErbB4 include betacellulin, epiregulin, HB-EGF, NRG-2, NRG-3, NRG-4 and heregulins. The ErbB ligand may also be a synthetic ErbB ligand.

The synthetic ligand may be specific for a particular ErbB receptor, or may recognize particular ErbB receptor complexes. An example of a synthetic ligand is the synthetic heregulin/EGF chimera heregulin (see, for example, Jones et al., (1999) *FEBS Letters*, 447:227-231, which is incorporated by reference).

"Heregulin" (HRG) refers to a polypeptide encoded by the heregulin gene product as disclosed in U.S. Pat. No. 5,641,869 or Marchionni et al., *Nature*, 362:312-318 (1993). Examples of heregulins include heregulin- α , heregulin- β 1, heregulin- β 2 and heregulin- β 3 (Holmes et al., *Science*, 256:1205-1210 (1992); and U.S. Pat. No. 5,641,869); neu differentiation factor (NDF) (Peles et al., *Cell* 69: 205-216 (1992)); acetylcholine receptor-inducing activity (ARIA)

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(Falls et al. (1993) *Cell* 72:801-815); glial growth factors (GGFs) (Marchionni et al., *Nature*, 362:312-318 (1993)); sensory and motor neuron derived factor (SMDF) (Ho et al., *J. Biol. Chem.*, 270:14523-14532 (1995)); γ -heregulin (Schaefer et al., *Oncogene*, 15:1385-1394 (1997)). The term includes biologically active fragments and/or amino acid sequence variants of a native sequence HRG polypeptide, such as an EGF-like domain fragment thereof (e.g., HRG β 1177-244).

"ErbB hetero-oligomer" is a noncovalently associated oligomer comprising at least two different ErbB receptors. An "ErbB dimer" is a noncovalently associated oligomer that comprises two different ErbB receptors. Such complexes may form when a cell expressing two or more ErbB receptors is exposed to an ErbB ligand. ErbB oligomers, such as ErbB dimers, can be isolated by immunoprecipitation and analyzed by SDS-PAGE as described in Sliwkowski et al., *J. Biol. Chem.*, 269(20):14661-14665 (1994), for example. Examples of such ErbB hetero-oligomers include EGFR-ErbB2 (also referred to as HER1/HER2), ErbB2-ErbB3 (HER2/HER3) and ErbB3-ErbB4 (HER3/HER4) complexes. Moreover, the ErbB hetero-oligomer may comprise two or more ErbB2 receptors combined with a different ErbB receptor, such as ErbB3, ErbB4 or EGFR (ErbB1). Other proteins, such as a cytokine receptor subunit (e.g., gp130) may be included in the hetero-oligomer.

A "native sequence" polypeptide is one which has the same amino acid sequence as a polypeptide, e.g., tumor-associated antigen receptor, derived from nature. Such native sequence polypeptides can be isolated from nature or can be produced by recombinant or synthetic means. Thus, a native sequence polypeptide can have the amino acid sequence of naturally-occurring human polypeptide, murine polypeptide, or polypeptide from any other mammalian species.

The term "amino acid sequence variant" refers to polypeptides having amino acid sequences that differ to some extent from a native sequence polypeptide. Ordinarily, amino acid sequence variants will possess at least about 70% homology with at least one receptor binding domain of a native ligand, or with at least one ligand binding domain of a native receptor, such as a tumor-associated antigen, and preferably, they will be at least about 80%, more preferably, at least about 90% homologous with such receptor or ligand binding domains. The amino acid sequence variants possess substitutions, deletions, and/or insertions at certain positions within the amino acid sequence of the native amino acid sequence.

"Sequence identity" is defined as the percentage of residues in the amino acid sequence variant that are identical after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Methods and computer programs for the alignment are well known in the art. One such computer program is "Align 2," authored by Genentech, Inc., which was filed with user documentation in the United States Copyright Office, Washington, D.C. 20559, on Dec. 10, 1991.

"Antibody-dependent cell-mediated cytotoxicity" and "ADCC" refer to a cell-mediated reaction in which non-specific cytotoxic cells that express Fc receptors (FcRs) (e.g., Natural Killer (NK) cells, neutrophils, and macrophages) recognize bound antibody on a target cell and subsequently cause lysis of the target cell. The primary cells for mediating ADCC, NK cells, express Fc γ RIII only, whereas monocytes express Fc γ RI, Fc γ RII and Fc γ RIII. FcR expression on hematopoietic cells in summarized is Table 3 on page 464 of Ravetch and Kinet, (1991) *Annu. Rev. Immunol.*, 9:457-92.

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To assess ADCC activity of a molecule of interest, an in vitro ADCC assay, such as that described in U.S. Pat. No. 5,500,362 or 5,821,337 may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed in vivo, e.g., in a animal model such as that disclosed in Clynes et al., *Prco. Natl. Acad. Sci. USA*, 95:652-656 (1998).

The terms "Fc receptor" or "FcR" are used to describe a receptor that binds to the Fc region of an antibody. The preferred FcR is a native sequence human FcR. Moreover, a preferred FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the Fc γ RI, Fc γ RII, and Fc γ RIII subclasses, including allelic variants and alternatively spliced forms of these receptors. Fc γ RII receptors include Fc γ RIIA (an "activating receptor") and Fc γ RIIB (an "inhibiting receptor"), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor Fc γ RIIA contains an immunoreceptor tyrosine-based activation motif (ITAM) in its cytoplasmic domain. Inhibiting receptor Fc γ RIIB contains an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic domain. (See review M. in Daëron, *Annu. Rev. Immunol.*, 15:203-234 (1997)). FcRs are reviewed in Ravetch and Kinet, *Annu. Rev. Immunol.*, 9:457-92 (1991); Capel et al., *Immunomethods*, 4:25-34 (1994); and de Haas et al., *J. Lab. Clin. Med.*, 126:330-41 (1995). Other FcRs, including those to be identified in the future, are encompassed by the term "FcR" herein. The term also includes the neonatal receptor, FcRn, which is responsible for the transfer of maternal IgGs to the fetus. (Guyer et al., *J. Immunol.*, 117:587 (1976) and Kim et al., *J. Immunol.*, 24:249 (1994)).

"Complement dependent cytotoxicity" or "CDC" refers to the ability of a molecule to lyse a target in the presence of complement. The complement activation pathway is initiated by the binding of the first component of the complement system (C1q) to a molecule (e.g., an antibody) complexed with a cognate antigen. To assess complement activation, a CDC assay, e.g., as described in Gazzano-Santoro et al., *J. Immunol. Methods*, 202:163 (1996), may be performed.

The term "variable" refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called hypervariable regions both in the light chain and the heavy chain variable domains. The more highly conserved portions of variable domains are called the framework regions (FRs). The variable domains of native heavy and light chains each comprise four FRs, largely adopting a β -sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases forming part of, the β -sheet structure. The hypervariable regions in each chain are held together in close proximity by the FRs and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat et al. (1991) *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md.). The constant domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody dependent cellular cytotoxicity (ADCC).

The term "hypervariable region" when used herein refers to the amino acid residues of an antibody which are respon-

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sible for antigen-binding. The hypervariable region generally comprises amino acid residues from a "complementarity determining region" or "CDR" (e.g., residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the light chain variable domain and 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy chain variable domain; Kabat et al. supra) and/or those residues from a "hypervariable loop" (e.g., residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the light chain variable domain and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the heavy chain variable domain; Chothia and Lesk (1987) *J. Mol. Biol.*, 196:901-917). "Framework Region" or "FR" residues are those variable domain residues other than the hypervariable region residues as herein defined.

Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen-binding sites and is still capable of cross-linking antigen.

"Fv" is the minimum antibody fragment which contains a complete antigen-recognition and antigen-binding site. This region consists of a dimer of one heavy chain and one light chain variable domain in tight, non-covalent association. It is in this configuration that the three hypervariable regions of each variable domain interact to define an antigen-binding site on the surface of the VH-VL dimer. Collectively, the six hypervariable regions confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three hypervariable regions specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

The Fab fragment also contains the constant domain of the light chain and the first constant domain (CH_1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain CH_1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear at least one free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

The "light chains" of antibodies from any vertebrate species can be assigned to one of two clearly distinct types, called kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains.

"Single-chain Fv" or "scFv" antibody fragments comprise the VH and VL domains of antibody, wherein these domains are present in a single polypeptide chain. Preferably, the Fv polypeptide further comprises a polypeptide linker between the VH and VL domains which enables the scFv to form the desired structure for antigen binding. For a review of scFv, see Plückthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenberg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a variable heavy domain (VH) connected to a variable light domain (VL) in the same polypeptide chain (VH-VL). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described

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more fully in, for example, EP 404,097; WO 93/11161; and Hollinger et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:6444-6448.

"Humanized" forms of non-human (e.g., rodent) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a hypervariable region of the recipient are replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and capacity. In some instances, framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin sequence. The humanized antibody optionally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones et al. (1986) *Nature*, 321:522-525; Riechmann et al. (1988) *Nature* 332:323-329; and Presta, (1992) *Curr. Op. Struct. Biol.*, 2:593-596.

Humanized anti-ErbB2 antibodies include huMAb4D5-1, huMAb4D5-2, huMAb4D5-3, huMAb4D5-4, huMAb4D5-5, huMAb4D5-6, huMAb4D5-7 and huMAb4D5-8)(HERCEPTIN® as described in Table 3 of U.S. Pat. No. 5,821,337 expressly incorporated herein by reference; humanized 520C₉ (WO 93/21319) and humanized 2C₄ antibodies as described herein below.

An "isolated" antibody is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In preferred embodiments, the antibody will be purified (1) to greater than 95% by weight of antibody as determined by the Lowry method, and most preferably more than 99% by weight, (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under reducing or nonreducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes the antibody in situ within recombinant cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

An antibody "which binds" an antigen of interest is one capable of binding that antigen with sufficient affinity such that the antibody is useful in targeting a cell expressing the antigen.

An antibody which "induces apoptosis" is one which induces programmed cell death as determined by binding of annexin V, fragmentation of DNA, cell shrinkage, dilation of endoplasmic reticulum, cell fragmentation, and/or formation of membrane vesicles (called apoptotic bodies). The cell is a tumor cell, e.g., a breast, ovarian, stomach, endometrial, salivary gland, lung, kidney, colon, thyroid, pancreatic or bladder cell. Various methods are available for evaluating

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the cellular events associated with apoptosis. For example, phosphatidyl serine (PS) translocation can be measured by annexin binding; DNA fragmentation can be evaluated through DNA laddering; and nuclear/chromatin condensation along with DNA fragmentation can be evaluated by any increase in hypodiploid cells.

A “disorder” is any condition that would benefit from treatment of the present invention. This includes chronic and acute disorders or diseases including those pathological conditions which predispose the mammal to the disorder in question. Non-limiting examples of disorders to be treated herein include benign and malignant tumors; leukemia and lymphoid malignancies, in particular breast, ovarian, stomach, endometrial, salivary gland, lung, kidney, colon, thyroid, pancreatic, prostate or bladder cancer; neuronal, glial, astrocytic, hypothalamic and other glandular, macrophagal, epithelial, stromal and blastocoele disorders; and inflammatory, angiogenic and immunologic disorders.

The term “therapeutically effective amount” refers to an amount of a drug effective to treat a disease or disorder in a mammal. In the case of cancer, the therapeutically effective amount of the drug may reduce the number of cancer cells; reduce the tumor size; inhibit (i.e., slow to some extent and preferably stop) cancer cell infiltration into peripheral organs; inhibit (i.e., slow to some extent and preferably stop) tumor metastasis; inhibit, to some extent, tumor growth; and/or relieve to some extent one or more of the symptoms associated with the cancer. To the extent the drug may prevent growth and/or kill existing cancer cells, it may be cytostatic and/or cytotoxic. For cancer therapy, efficacy can, for example, be measured by assessing the time to disease progression (TTP) and/or determining the response rate (RR).

The term “substantial amount” refers to a majority, i.e., >50% of a population, of a collection or a sample.

The term “intracellular metabolite” refers to a compound resulting from a metabolic process or reaction inside a cell on an antibody drug conjugate (ADC). The metabolic process or reaction may be an enzymatic process such as proteolytic cleavage of a peptide linker of the ADC, or hydrolysis of a functional group such as a hydrazone, ester, or amide. Intracellular metabolites include, but are not limited to, antibodies and free drug which have undergone intracellular cleavage after entry, diffusion, uptake or transport into a cell.

The terms “intracellularly cleaved” and “intracellular cleavage” refer to a metabolic process or reaction inside a cell on an Drug-Ligand Conjugate, a Drug-Linker-Ligand Conjugate, an antibody drug conjugate (ADC) or the like whereby the covalent attachment, e.g., the linker, between the drug moiety (D) and the antibody (Ab) is broken, resulting in the free drug dissociated from the antibody inside the cell. The cleaved moieties of the Drug-Ligand Conjugate, a Drug-Linker-Ligand Conjugate or ADC are thus intracellular metabolites.

The term “bioavailability” refers to the systemic availability (i.e., blood/plasma levels) of a given amount of drug administered to a patient. Bioavailability is an absolute term that indicates measurement of both the time (rate) and total amount (extent) of drug that reaches the general circulation from an administered dosage form.

The term “cytotoxic activity” refers to a cell-killing, cytostatic or anti-proliferation effect of an antibody drug conjugate compound or an intracellular metabolite of an antibody drug conjugate compound. Cytotoxic activity may

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be expressed as the IC50 value which is the concentration (molar or mass) per unit volume at which half the cells survive.

The terms “cancer” and “cancerous” refer to or describe the physiological condition in mammals that is typically characterized by unregulated cell growth. A “tumor” comprises one or more cancerous cells. Examples of cancer include, but are not limited to, carcinoma, lymphoma, blastoma, sarcoma, and leukemia or lymphoid malignancies. More particular examples of such cancers include squamous cell cancer (e.g., epithelial squamous cell cancer), lung cancer including small-cell lung cancer, non-small cell lung cancer (“NSCLC”), adenocarcinoma of the lung and squamous carcinoma of the lung, cancer of the peritoneum, hepatocellular cancer, gastric or stomach cancer including gastrointestinal cancer, pancreatic cancer, glioblastoma, cervical cancer, ovarian cancer, liver cancer, bladder cancer, hepatoma, breast cancer, colon cancer, rectal cancer, colorectal cancer, endometrial or uterine carcinoma, salivary gland carcinoma, kidney or renal cancer, prostate cancer, vulval cancer, thyroid cancer, hepatic carcinoma, anal carcinoma, penile carcinoma, as well as head and neck cancer.

An “ErbB2-expressing cancer” is one which produces sufficient levels of ErbB2 at the surface of cells thereof, such that an anti-ErbB2 antibody can bind thereto and have a therapeutic effect with respect to the cancer.

A cancer “characterized by excessive activation” of an ErbB2 receptor is one in which the extent of ErbB2 receptor activation in cancer cells significantly exceeds the level of activation of that receptor in non-cancerous cells of the same tissue type. Such excessive activation may result from overexpression of the ErbB2 receptor and/or greater than normal levels of an ErbB2 ligand available for activating the ErbB2 receptor in the cancer cells. Such excessive activation may cause and/or be caused by the malignant state of a cancer cell. In some embodiments, the cancer will be subjected to a diagnostic or prognostic assay to determine whether amplification and/or overexpression of an ErbB2 receptor is occurring which results in such excessive activation of the ErbB2 receptor. Alternatively, or additionally, the cancer may be subjected to a diagnostic or prognostic assay to determine whether amplification and/or overexpression an ErbB2 ligand is occurring in the cancer which attributes to excessive activation of the receptor. In a subset of such cancers, excessive activation of the receptor may result from an autocrine stimulatory pathway.

A cancer which “overexpresses” an ErbB2 receptor is one which has significantly higher levels of an ErbB2 receptor at the cell surface thereof, compared to a noncancerous cell of the same tissue type. Such overexpression may be caused by gene amplification or by increased transcription or translation. ErbB2 receptor overexpression may be determined in a diagnostic or prognostic assay by evaluating increased levels of the ErbB2 protein present on the surface of a cell (e.g., via an immunohistochemistry assay; IHC). Alternatively, or additionally, one may measure levels of ErbB2-encoding nucleic acid in the cell, e.g., via fluorescent in situ hybridization (FISH; see WO 98/45479), southern blotting, or polymerase chain reaction (PCR) techniques, such as real time quantitative PCR (RT-PCR). Overexpression of the ErbB2 ligand, may be determined diagnostically by evaluating levels of the ligand (or nucleic acid encoding it) in the patient, e.g., in a tumor biopsy or by various diagnostic assays such as the IHC, FISH, southern blotting, PCR or in vivo assays described above. One may also study ErbB2 receptor overexpression by measuring shed antigen (e.g., ErbB2 extracellular domain) in a biological fluid such as

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serum (see, e.g., U.S. Pat. No. 4,933,294; WO 91/05264; U.S. Pat. No. 5,401,638; and Sias et al., (1990) *J. Immunol. Methods*, 132: 73-80). Aside from the above assays, various other in vivo assays are available to the skilled practitioner. For example, one may expose cells within the body of the patient to an antibody which is optionally labeled with a detectable label, e.g., a radioactive isotope, and binding of the antibody to cells in the patient can be evaluated, e.g., by external scanning for radioactivity or by analyzing a biopsy taken from a patient previously exposed to the antibody.

The tumors overexpressing HER2 are rated by immunohistochemical scores corresponding to the number of copies of HER2 molecules expressed per cell, and can be determined biochemically: 0=0-10,000 copies/cell, 1+=at least about 200,000 copies/cell, 2+=at least about 500,000 copies/cell, 3+=about 1-2 $\times 10^6$ copies/cell. Overexpression of HER2 at the 3+ level, which leads to ligand-independent activation of the tyrosine kinase (Hudziak et al., (1987) *Proc. Natl. Acad. Sci. USA*, 84:7159-7163), occurs in approximately 30% of breast cancers, and in these patients, relapse-free survival and overall survival are diminished (Slamon et al., (1989) *Science*, 244:707-712; Slamon et al., (1987) *Science*, 235:177-182).

Conversely, a cancer which is "not characterized by overexpression of the ErbB2 receptor" is one which, in a diagnostic assay, does not express higher than normal levels of ErbB2 receptor compared to a noncancerous cell of the same tissue type.

The term "cytotoxic agent" as used herein refers to a substance that inhibits or prevents the function of cells and/or causes destruction of cells. The term is intended to include radioactive isotopes (e.g., ^{211}At , ^{131}I , ^{125}I , ^{90}Y , ^{186}Re , ^{188}Re , ^{153}Sm , ^{212}Bi , ^{32}P , ^{60}Co , and radioactive isotopes of Lu), chemotherapeutic agents, and toxins such as small molecule toxins or enzymatically active toxins of bacterial, fungal, plant or animal origin, including synthetic analogs and derivatives thereof. In one aspect, the term is not intended to include radioactive isotopes.

A "chemotherapeutic agent" is a chemical compound useful in the treatment of cancer. Examples of chemotherapeutic agents include alkylating agents such as thiopeta and CYTOXAN® cyclophosphamide; alkyl sulfonates such as busulfan, improsulfan and piposulfan; aziridines such as benzodopa, carboquone, meturedopa, and uredopa; ethylenimines and methylamelamines including altretamine, triethylenemelamine, triethylenephosphoramide, triethylenethiophosphoramide and trimethylolomelamine; TLK 286 (TELCYTA™); acetogenins (especially bullatacin and bullatacinone); delta-9-tetrahydrocannabinol (dronabinol, MARINOL®); beta-lapachone; lapachol; colchicines; betulinic acid; a camptothecin (including the synthetic analogue topotecan)(HYCAMTIN®, CPT-11 (irinotecan, CAMPTOSAR®), acetylcamptothecin, scopoletin, and 9-aminocamptothecin); bryostatins; callistatin; CC-1065 (including its adozelesin, carzelesin and bizelesin synthetic analogues); podophyllotoxin;

podophyllinic acid; teniposide; cryptophycins (particularly cryptophycin 1 and cryptophycin 8);

dolastatin; duocarmycin (including the synthetic analogues, KW-2189 and CB1-TM1);

eleutherobin; pancratiastatin; a sarcodictyin; spongistatin; nitrogen mustards such as chlorambucil, chlornaphazine, cholophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, uracil mustard; nitrosureas such as carmustine, chlorozotocin, fotemustine, lomustine, nimustine, and ranimustine;

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bisphosphonates, such as clodronate; antibiotics such as the enediyne antibiotics (e. g., calicheamicin, especially calicheamicin gammaII and calicheamicin omegaI (see, e.g., Agnew, *Chem Intl. Ed. Engl.*, 33: 183-186 (1994)) and anthracyclines such as annamycin, AD 32, alcarubicin, daunorubicin, dexrazoxane, DX-52-1, epirubicin, GPX-100, idarubicin, KRN5500, menogaril, dynemicin, including dynemicin A, an esperamicin, neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromophores, aclacinomysins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabacin, carminomycin, carzinophilin, chromomycins, dactinomycin, detorubicin, 6-diazo-5-oxo-L-norleucine, ADRIAMYCIN® doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin, liposomal doxorubicin, and deoxydoxorubicin), esorubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, potfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, and zorubicin; folic acid analogues such as denopterin, pteropterin, and trimetrexate; purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, and thioguanine; pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, and floxuridine; androgens such as calusterone, dromostanolone propionate, epitostanol, mepitiostane, and testolactone; antiadrenals such as aminoglutethimide, mitotane, and trilostane; folic acid replenisher such as folinic acid (leucovorin); aceglatone; anti-folate anti-neoplastic agents such as ALIMTA®, LY231514 pemetrexed, dihydrofolate reductase inhibitors such as methotrexate, anti-metabolites such as 5-fluorouracil (5-FU) and its prodrugs such as UFT, S-1 and capecitabine, and thymidylate synthase inhibitors and glycinamide ribonucleotide formyltransferase inhibitors such as raltitrexed (TOMUDEX®, TDX); inhibitors of dihydropyrimidine dehydrogenase such as eniluracil; aldophosphamide glycoside; aminolevulinic acid; amasacrine; bestabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elfornithine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; lonidainine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidanmol; nitraerine; pentostatin; phenamet; pirarubicin; losoxantrone; 2-ethylhydrazide; procarbazine; PSK® polysaccharide complex (JHS Natural Products, Eugene, Oreg.); razoxane; rhizoxine; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2"-trichlorotriethylamine; trichothecenes (especially T-2 toxin, verracurin A, roridin A and anguidine); urethan; vindesine (ELDISINE®, FILDESIN®); dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiopeta; taxoids and taxanes, e.g., TAXOL® paclitaxel (Bristol-Myers Squibb Oncology, Princeton, N.J.), ABRAXANE™ Cremophor-free, albumin-engineered nanoparticle formulation of paclitaxel (American Pharmaceutical Partners, Schaumburg, Ill.), and TAXOTERE® doxetaxel (Rhone-Poulenc Rorer, Antony, France); chlorambucil; gemcitabine(GEMZAR®); 6-thioguanine; mercaptopurine; platinum; platinum analogs or platinum-based analogs such as cisplatin, oxaliplatin and carboplatin; vinblastine(VELBAN®; etoposide (VP-16); ifosfamide; mitoxantrone; vincristine)(ONCOVIN®; vinca alkaloid; vinorelbine)(NAVELBINE®; novantrone; edatrexate; daunomycin; aminopterin; xeloda; ibandronate; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DMFO); retinoids such as retinoic acid; pharmaceutically acceptable salts, acids or derivatives of any of the above; as

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well as combinations of two or more of the above such as CHOP, an abbreviation for a combined therapy of cyclophosphamide, doxorubicin, vincristine, and prednisolone, and FOLFOX, an abbreviation for a treatment regimen with oxaliplatin (ELOXATIN™) combined with 5-FU and leucovorin.

Also included in this definition are anti-hormonal agents that act to regulate or inhibit hormone action on tumors such as anti-estrogens and selective estrogen receptor modulators (SERMs), including, for example, tamoxifen (including NOLVADEX® tamoxifen), raloxifene, droloxifene, 4-hydroxytamoxifen, trioxifene, keoxifene, LY117018, onapristone, and FARESTON® toremifene; aromatase inhibitors that inhibit the enzyme aromatase, which regulates estrogen production in the adrenal glands, such as, for example, 4(5)-imidazoles, aminoglutethimide, MEGASE® megestrol acetate, AROMASIN® exemestane, formestane, fadrozole, RIVISOR® vorozole, FEMARA® letrozole, and ARIMIDEX® anastrozole; and anti-androgens such as flutamide, nilutamide, bicalutamide, leuprolide, and goserelin; as well as troxacitabine (a 1,3-dioxolane nucleoside cytosine analog); antisense oligonucleotides, particularly those that inhibit expression of genes in signaling pathways implicated in adherent cell proliferation, such as, for example, PKC- α , Raf, H-Ras, and epidermal growth factor receptor (EGF-R); vaccines such as gene therapy vaccines, for example, ALLOVECTIN® vaccine, LEUVECTIN® vaccine, and VAXID® vaccine; PROLEUKIN® rIL-2; LURTOTECAN® topoisomerase 1 inhibitor; ABARELIX® rmRH; and pharmaceutically acceptable salts, acids or derivatives of any of the above.

As used herein, the term “EGFR-targeted drug” refers to a therapeutic agent that binds to EGFR and, optionally, inhibits EGFR activation. Examples of such agents include antibodies and small molecules that bind to EGFR. Examples of antibodies which bind to EGFR include MAB 579 (ATCC CRL HB 8506), MAb 455 (ATCC CRL HB8507), MAb 225 (ATCC CRL 8508), MAb 528 (ATCC CRL 8509) (see, U.S. Pat. No. 4,943,533, Mendelsohn et al.) and variants thereof, such as chimerized 225 (C₂₂₅ or Cetuximab; ERBITUX®) and reshaped human 225 (H225) (see, WO 96/40210, Imclone Systems Inc.); antibodies that bind type II mutant EGFR (U.S. Pat. No. 5,212,290); humanized and chimeric antibodies that bind EGFR as described in U.S. Pat. No. 5,891,996; and human antibodies that bind EGFR, such as ABX-EGF (see WO 98/50433, Abgenix). The anti-EGFR antibody may be conjugated with a cytotoxic agent, thus generating an immunoconjugate (see, e.g., EP 659, 439A2, Merck Patent GmbH). Examples of small molecules that bind to EGFR include ZD1839 or Gefitinib (IRESSA™; Astra Zeneca), Erlotinib HCl (CP-358774, TARCEVA™; Genentech/OSI) and AG1478, AG1571 (SU 5271; Sugen).

A “tyrosine kinase inhibitor” is a molecule which inhibits to some extent tyrosine kinase activity of a tyrosine kinase such as an ErbB receptor. Examples of such inhibitors include the EGFR-targeted drugs noted in the preceding paragraph as well as quinazolines such as PD 153035, 4-(3-chloroanilino) quinazoline, pyridopyrimidines, pyrimidopyrimidines, pyrrolopyrimidines, such as CGP 59326, CGP 60261 and CGP 62706, and pyrazolopyrimidines, 4-(phenylamino)-7H-pyrrolo[2,3-d]pyrimidines, curcumin (diferuloyl methane, 4,5-bis(4-fluoroanilino)phthalimide), tyrphostines containing nitrothiophene moieties; PD-0183805 (Warner-Lambert); antisense molecules (e.g., those that bind to ErbB-encoding nucleic acid); quinoxalines (U.S. Pat. No. 5,804,396); tyrphostins (U.S. Pat. No. 5,804, 396); ZD6474 (Astra Zeneca); PTK-787 (Novartis/Schering

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AG); pan-ErbB inhibitors such as CI-1033 (Pfizer); Affinitac (ISIS 3521; Isis/Lilly); Imatinib mesylate (Gleevec; Novartis); PKI 166 (Novartis); GW2016 (Glaxo SmithKline); CI-1033 (Pfizer); EKB-569 (Wyeth); Semaxanib (Sugen); ZD6474 (AstraZeneca); PTK-787 (Novartis/Schering AG); INC-1C₁₁ (Imclone); or as described in any of the following patent publications: U.S. Pat. No. 5,804,396; WO 99/09016 (American Cyanamid); WO 98/43960 (American Cyanamid); WO 97/38983 (Warner Lambert); WO 99/06378 (Warner Lambert); WO 99/06396 (Warner Lambert); WO 96/30347 (Pfizer, Inc); WO 96/33978 (Zeneca); WO 96/3397 (Zeneca); and WO 96/33980 (Zeneca).

An “anti-angiogenic agent” refers to a compound which blocks, or interferes with to some degree, the development of blood vessels. The anti-angiogenic factor may, for instance, be a small molecule or antibody that binds to a growth factor or growth factor receptor involved in promoting angiogenesis. In one embodiment, the anti-angiogenic factor is an antibody that binds to Vascular Endothelial Growth Factor (VEGF).

The term “cytokine” is a generic term for proteins released by one cell population which act on another cell as intercellular mediators. Examples of such cytokines are lymphokines, monokines, and traditional polypeptide hormones. Included among the cytokines are growth hormone such as human growth hormone, N-methionyl human growth hormone, and bovine growth hormone; parathyroid hormone; thyroxine; insulin; proinsulin; relaxin; prolactin; glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH); hepatic growth factor; fibroblast growth factor; prolactin; placental lactogen; tumor necrosis factor- α and - β ; mullerian-inhibiting substance; mouse gonadotropin-associated peptide; inhibin; activin; vascular endothelial growth factor; integrin; thrombopoietin (TPO); nerve growth factors such as NGF- β ; platelet-growth factor; transforming growth factors (TGFs) such as TGF- α and TGF- β ; insulin-like growth factor-I and -II; erythropoietin (EPO); osteoinductive factors; interferons such as interferon- α , - β , and - γ ; colony stimulating factors (CSFs) such as macrophage-CSF (M-CSF); granulocyte-macrophage-CSF (GM-CSF); and granulocyte-CSF (G-CSF); interleukins (ILs) such as IL-1, IL-1 α , IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12; a tumor necrosis factor such as TNF- α or TNF- β ; and other polypeptide factors including LIF and kit ligand (KL). As used herein, the term cytokine includes proteins from natural sources or from recombinant cell culture and biologically active equivalents of the native sequence cytokines.

The term “prodrug” as used in this application refers to a precursor or derivative form of a pharmaceutically active substance that is less cytotoxic to tumor cells compared to the parent drug and is capable of being enzymatically or hydrolytically activated or converted into the more active parent form. See, e.g., Wilman, “Prodrugs in Cancer Chemotherapy” Biochemical Society Transactions, 14, pp. 375-382, 615th Meeting Belfast (1986) and Stella et al., “Prodrugs: A Chemical Approach to Targeted Drug Delivery,” *Directed Drug Delivery*, Borchardt et al., (ed.), pp. 247-267, Humana Press (1985). The prodrugs of this invention include, but are not limited to, phosphate-containing prodrugs, thiophosphate-containing prodrugs, sulfate-containing prodrugs, peptide-containing prodrugs, D-amino acid-modified prodrugs, glycosylated prodrugs, β -lactam-containing prodrugs, optionally substituted phenoxyacetamide-containing prodrugs or optionally substituted phenylacetamide-containing prodrugs, 5-fluorocytosine and other 5-fluorou-

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ridine prodrugs which can be converted into the more active cytotoxic free drug. Examples of cytotoxic drugs that can be derivatized into a prodrug form for use in this invention include, but are not limited to, those chemotherapeutic agents described above.

A "liposome" is a small vesicle composed of various types of lipids, phospholipids and/or surfactant which is useful for delivery of a drug (such as including the anti-CD30, CD40, CD70 or Lewis Y antibodies and, optionally, a chemotherapeutic agent) to a mammal. The components of the liposome are commonly arranged in a bilayer formation, similar to the lipid arrangement of biological membranes.

The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, contraindications and/or warnings concerning the use of such therapeutic products.

An "isolated" nucleic acid molecule is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the natural source of the antibody nucleic acid. An isolated nucleic acid molecule is other than in the form or setting in which it is found in nature. Isolated nucleic acid molecules therefore are distinguished from the nucleic acid molecule as it exists in natural cells. However, an isolated nucleic acid molecule includes a nucleic acid molecule contained in cells that ordinarily express the antibody where, for example, the nucleic acid molecule is in a chromosomal location different from that of natural cells.

The expression "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding site. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

A nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking can be accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers can be used in accordance with conventional practice.

As used herein, the expressions "cell," "cell line," and "cell culture" are used interchangeably and all such designations include progeny. Thus, the words "transformants" and "transformed cells" include the primary subject cell and cultures derived therefrom without regard for the number of transfers. It is also understood that all progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. Mutant progeny that have the same function or biological activity as screened for in the originally transformed cell are included. Where distinct designations are intended, it will be clear from the context.

An "autoimmune disease" herein is a disease or disorder arising from and directed against an individual's own tissues or a co-segregate or manifestation thereof or resulting con-

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dition therefrom. Examples of autoimmune diseases or disorders include, but are not limited to arthritis (rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis, psoriatic arthritis, and ankylosing spondylitis), psoriasis, dermatitis including atopic dermatitis; chronic idiopathic urticaria, including chronic autoimmune urticaria, polymyositis/dermatomyositis, toxic epidermal necrolysis, systemic sclerosis and scleroderma, responses associated with inflammatory bowel disease (IBD) (Crohn's disease, ulcerative colitis), and IBD with co-segregate of pyoderma gangrenosum, erythema nodosum, primary sclerosing cholangitis, and/or episcleritis), respiratory distress syndrome, including adult respiratory distress syndrome (ARDS), meningitis, IgE-mediated diseases such as anaphylaxis and allergic rhinitis, encephalitis such as Rasmussen's encephalitis, uveitis, colitis such as microscopic colitis and collagenous colitis, glomerulonephritis (GN) such as membranous GN, idiopathic membranous GN, membranous proliferative GN (MPGN), including Type I and Type II, and rapidly progressive GN, allergic conditions, eczema, asthma, conditions involving infiltration of T cells and chronic inflammatory responses, atherosclerosis, autoimmune myocarditis, leukocyte adhesion deficiency, systemic lupus erythematosus (SLE) such as cutaneous SLE, lupus (including nephritis, cerebritis, pediatric, non-renal, discoid, alopecia), juvenile onset diabetes, multiple sclerosis (MS) such as spino-optical MS, allergic encephalomyelitis, immune responses associated with acute and delayed hypersensitivity mediated by cytokines and T-lymphocytes, tuberculosis, sarcoidosis, granulomatosis including Wegener's granulomatosis, agranulocytosis, vasculitis (including Large Vessel vasculitis (including Polymyalgia Rheumatica and Giant Cell (Takayasu's) Arteritis), Medium Vessel vasculitis (including Kawasaki's Disease and Polyarteritis Nodosa), CNS vasculitis, and ANCA-associated vasculitis, such as Churg-Strauss vasculitis or syndrome (CSS), aplastic anemia, Coombs positive anemia, Diamond Blackfan anemia, immune hemolytic anemia including autoimmune hemolytic anemia (AIHA), pernicious anemia, pure red cell aplasia (PRCA), Factor VIII deficiency, hemophilia A, autoimmune neutropenia, pancytopenia, leukopenia, diseases involving leukocyte diapedesis, CNS inflammatory disorders, multiple organ injury syndrome, myasthenia gravis, antigen-antibody complex mediated diseases, anti-glomerular basement membrane disease, anti-phospholipid antibody syndrome, allergic neuritis, Bechet disease, Castleman's syndrome, Goodpasture's Syndrome, Lambert-Eaton Myasthenic Syndrome, Reynaud's syndrome, Sjorgen's syndrome, Stevens-Johnson syndrome, solid organ transplant rejection (including pre-treatment for high panel reactive antibody titers, IgA deposit in tissues, and rejection arising from renal transplantation, liver transplantation, intestinal transplantation, cardiac transplantation, etc.), graft versus host disease (GVHD), pemphigoid bullous, pemphigus (including vulgaris, foliaceus, and pemphigus mucus-membrane pemphigoid), autoimmune polyendocrinopathies, Reiter's disease, stiff-man syndrome, immune complex nephritis, IgM polyneuropathies or IgM mediated neuropathy, idiopathic thrombocytopenic purpura (ITP), thrombotic thrombocytopenic purpura (TTP), thrombocytopenia (as developed by myocardial infarction patients, for example), including autoimmune thrombocytopenia, autoimmune disease of the testis and ovary including autoimmune orchitis and oophoritis, primary hypothyroidism; autoimmune endocrine diseases including autoimmune thyroiditis, chronic thyroiditis (Hashimoto's Thyroiditis), subacute thyroiditis, idiopathic hypothyroidism, Addison's disease, Grave's disease, auto-

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immune polyglandular syndromes (or polyglandular endocrinopathy syndromes), Type I diabetes also referred to as insulin-dependent diabetes mellitus (IDDM), including pediatric IDDM, and Sheehan's syndrome; autoimmune hepatitis, Lymphoid interstitial pneumonitis (HIV), bronchiolitis obliterans (non-transplant) vs NSIP, Guillain-Barré Syndrome, Berger's Disease (IgA nephropathy), primary biliary cirrhosis, celiac sprue (gluten enteropathy), refractory sprue with co-segregate dermatitis herpetiformis, cryoglobulinemia, amyotrophic lateral sclerosis (ALS; Lou Gehrig's disease), coronary artery disease, autoimmune inner ear disease (AIED), autoimmune hearing loss, opso-clonus myoclonus syndrome (OMS), polychondritis such as refractory polychondritis, pulmonary alveolar proteinosis, amyloidosis, giant cell hepatitis, scleritis, monoclonal gammopathy of uncertain/unknown significance (MGUS), peripheral neuropathy, paraneoplastic syndrome, channelopathies such as epilepsy, migraine, arrhythmia, muscular disorders, deafness, blindness, periodic paralysis, and channelopathies of the CNS; autism, inflammatory myopathy, and focal segmental glomerulosclerosis (FSGS).

"Alkyl" is C_1 - C_{18} hydrocarbon containing normal, secondary, tertiary or cyclic carbon atoms. Examples are methyl (Me, $-CH_3$), ethyl (Et, $-CH_2CH_3$), 1-propyl (n-Pr, n-propyl, $-CH_2CH_2CH_3$), 2-propyl (i-Pr, i-propyl, $-CH(CH_3)_2$), 1-butyl (n-Bu, n-butyl, $-CH_2CH_2CH_2CH_3$), 2-methyl-1-propyl (i-Bu, i-butyl, $-CH_2CH(CH_3)_2$), 2-butyl (s-Bu, s-butyl, $-CH(CH_3)CH_2CH_3$), 2-methyl-2-propyl (t-Bu, t-butyl, $-C(CH_3)_3$), 1-pentyl (n-pentyl, $-CH_2CH_2CH_2CH_2CH_3$), 2-pentyl ($-CH(CH_3)CH_2CH_2CH_3$), 3-pentyl ($-CH(CH_2CH_3)_2$), 2-methyl-2-butyl ($-C(CH_3)_2CH_2CH_3$), 3-methyl-2-butyl ($-CH(CH_3)CH(CH_3)CH_2CH_3$), 3-methyl-1-butyl ($-CH_2CH_2CH(CH_3)CH_3$), 2-methyl-1-butyl ($-CH_2CH(CH_3)CH_2CH_3$), 1-hexyl ($-CH_2CH_2CH_2CH_2CH_2CH_3$), 2-hexyl ($-CH(CH_3)CH_2CH_2CH_2CH_3$), 3-hexyl ($-CH(CH_2CH_3)CH_2CH_2CH_3$), 2-methyl-2-pentyl ($-C(CH_3)_2CH_2CH_2CH_3$), 3-methyl-2-pentyl ($-CH(CH_3)CH(CH_3)CH_2CH_3$), 4-methyl-2-pentyl ($-CH(CH_3)CH_2CH(CH_3)_2$), 3-methyl-3-pentyl ($-C(CH_3)(CH_2CH_3)_2$), 2-methyl-3-pentyl ($-CH(CH_2CH_3)CH(CH_3)_2$), 2,3-dimethyl-2-butyl ($-C(CH_3)_2CH(CH_3)_2$), 3,3-dimethyl-2-butyl ($-CH(CH_3)C(CH_3)_3$).

"Alkenyl" is C_2 - C_{18} hydrocarbon containing normal, secondary, tertiary or cyclic carbon atoms with at least one site of unsaturation, i.e. a carbon-carbon, sp^2 double bond. Examples include, but are not limited to: ethylene or vinyl ($-CH=CH_2$), allyl ($-CH_2CH=CH_2$), cyclopentenyl ($-C_5H_7$), and 5-hexenyl ($-CH_2CH_2CH_2CH_2CH=CH_2$).

"Alkynyl" is C_2 - C_{18} hydrocarbon containing normal, secondary, tertiary or cyclic carbon atoms with at least one site of unsaturation, i.e. a carbon-carbon, sp triple bond. Examples include, but are not limited to: acetylenic ($-C\equiv CH$) and propargyl ($-CH_2C\equiv CH$).

"Alkylene" refers to a saturated, branched or straight chain or cyclic hydrocarbon radical of 1-18 carbon atoms, and having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent alkane. Typical alkylene radicals include, but are not limited to: methylene ($-CH_2-$), 1,2-ethyl ($-CH_2CH_2-$), 1,3-propyl ($-CH_2CH_2CH_2-$), 1,4-butyl ($-CH_2CH_2CH_2CH_2-$), and the like.

"Alkenylene" refers to an unsaturated, branched or straight chain or cyclic hydrocarbon radical of 2-18 carbon atoms, and having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two

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different carbon atoms of a parent alkene. Typical alkenylene radicals include, but are not limited to: 1,2-ethylene ($-CH=CH-$).

"Alkynylene" refers to an unsaturated, branched or straight chain or cyclic hydrocarbon radical of 2-18 carbon atoms, and having two monovalent radical centers derived by the removal of two hydrogen atoms from the same or two different carbon atoms of a parent alkyne. Typical alkynylene radicals include, but are not limited to: acetylene ($-C\equiv C-$), propargyl ($-CH_2C\equiv C-$), and 4-pentynyl ($-CH_2CH_2CH_2C\equiv CH-$).

"Aryl" means a monovalent aromatic hydrocarbon radical of 6-20 carbon atoms derived by the removal of one hydrogen atom from a single carbon atom of a parent aromatic ring system. Some aryl groups are represented in the exemplary structures as "Ar". Typical aryl groups include, but are not limited to, radicals derived from benzene, substituted benzene, naphthalene, anthracene, biphenyl, and the like.

"Arylalkyl" refers to an acyclic alkyl radical in which one of the hydrogen atoms bonded to a carbon atom, typically a terminal or sp^3 carbon atom, is replaced with an aryl radical. Typical arylalkyl groups include, but are not limited to, benzyl, 2-phenylethan-1-yl, 2-phenylethen-1-yl, naphthylmethyl, 2-naphthylethan-1-yl, 2-naphthylethen-1-yl, naphthobenzyl, 2-naphthophenylethan-1-yl and the like. The arylalkyl group comprises 6 to 20 carbon atoms, e.g., the alkyl moiety, including alkanyl, alkenyl or alkynyl groups, of the arylalkyl group is 1 to 6 carbon atoms and the aryl moiety is 5 to 14 carbon atoms.

"Heteroarylalkyl" refers to an acyclic alkyl radical in which one of the hydrogen atoms bonded to a carbon atom, typically a terminal or sp^3 carbon atom, is replaced with a heteroaryl radical. Typical heteroarylalkyl groups include, but are not limited to, 2-benzimidazolylmethyl, 2-furylethyl, and the like. The heteroarylalkyl group comprises 6 to 20 carbon atoms, e.g., the alkyl moiety, including alkanyl, alkenyl or alkynyl groups, of the heteroarylalkyl group is 1 to 6 carbon atoms and the heteroaryl moiety is 5 to 14 carbon atoms and 1 to 3 heteroatoms selected from N, O, P, and S. The heteroaryl moiety of the heteroarylalkyl group may be a monocycle having 3 to 7 ring members (2 to 6 carbon atoms or a bicycle having 7 to 10 ring members (4 to 9 carbon atoms and 1 to 3 heteroatoms selected from N, O, P, and S), for example: a bicyclo [4,5], [5,5], [5,6], or [6,6] system.

"Substituted alkyl", "substituted aryl", and "substituted arylalkyl" mean alkyl, aryl, and arylalkyl respectively, in which one or more hydrogen atoms are each independently replaced with a substituent. Typical substituents include, but are not limited to, $-X$, $-R$, $-OR$, $-SR$, $-S^+$, $-NR_2$, $-NR_3$, $=NR$, $-CX_3$, $-CN$, $-OCN$, $-SCN$, $-N=C=O$, $-NCS$, $-NO$, $-NO_2$, $=N_2$, $-N_3$, $NC(=O)R$, $-C(=O)R$, $-C(=O)NR_2$, $-SO_3H$, $-S(=O)_2R$, $-OS(=O)_2OR$, $-S(=O)_2NR$, $-S(=O)R$, $-OP(=O)(OR)_2$, $-P(=O)(OR)_2$, $-PO_3^-$, $-PO_3H_2$, $-C(=O)R$, $-C(=O)X$, $-C(=S)R$, $-CO_2R$, $-CO_2^-$, $-C(=S)OR$, $-C(=O)SR$, $-C(=S)SR$, $-C(=O)NR_2$, $-C(=S)NR_2$, $-C(=NR)NR_2$, where each X is independently a halogen: F, Cl, Br, or I; and each R is independently $-H$, C_2 - C_{18} alkyl, C_6 - C_{20} aryl, C_3 - C_{14} heterocycle, protecting group or prodrug moiety. Alkylene, alkenylene, and alkynylene groups as described above may also be similarly substituted.

"Heteroaryl" and "Heterocycle" refer to a ring system in which one or more ring atoms is a heteroatom, e.g., nitrogen, oxygen, and sulfur. The heterocycle radical comprises 1 to 20 carbon atoms and 1 to 3 heteroatoms selected from N, O,

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P, and S. A heterocycle may be a monocycle having 3 to 7 ring members (2 to 6 carbon atoms and 1 to 3 heteroatoms selected from N, O, P, and S) or a bicycle having 7 to 10 ring members (4 to 9 carbon atoms and 1 to 3 heteroatoms selected from N, O, P, and S), for example: a bicyclo [4,5], [5,5], [5,6], or [6,6] system.

Heterocycles are described in Paquette, Leo A.; "Principles of Modern Heterocyclic Chemistry" (W. A. Benjamin, New York, 1968), particularly Chapters 1, 3, 4, 6, 7, and 9; "The Chemistry of Heterocyclic Compounds, A series of Monographs" (John Wiley & Sons, New York, 1950 to present), in particular Volumes 13, 14, 16, 19, and 28; and *J. Am. Chem. Soc.* (1960) 82:5566.

Examples of heterocycles include by way of example and not limitation pyridyl, dihydropyridyl, tetrahydropyridyl (piperidyl), thiazolyl, tetrahydrothiophenyl, sulfur oxidized tetrahydrothiophenyl, pyrimidinyl, furanyl, thienyl, pyrrolyl, pyrazolyl, imidazolyl, tetrazolyl, benzofuranyl, thianaphthalenyl, indolyl, indolenyl, quinolinyl, isoquinolinyl, benzimidazolyl, piperidinyl, 4-piperidinyl, pyrrolidinyl, 2-pyrrolidinyl, pyrrolinyl, tetrahydrofuranyl, bis-tetrahydrofuranyl, tetrahydropyranyl, bis-tetrahydropyranyl, tetrahydroquinolinyl, tetrahydroisoquinolinyl, decahydroquinolinyl, octahydroisoquinolinyl, azocinyl, triazinyl, 6H-1,2,5-thiadiazinyl, 2H,6H-1,5,2-dithiazinyl, thienyl, thianthrenyl, pyranyl, isobenzofuranyl, chromenyl, xanthenyl, phenoxathinyl, 2H-pyrrolyl, isothiazolyl, isoxazolyl, pyrazinyl, pyridazinyl, indoliziny, isindolyl, 3H-indolyl, 1H-indazolyl, purinyl, 4H-quinoliziny, phthalazinyl, naphthyridinyl, quinoxalinyl, quinazolinyl, cinnolinyl, pteridinyl, 4aH-carbazolyl, carbazolyl, β -carbolinyl, phenanthridinyl, acridinyl, pyrimidinyl, phenanthrolinyl, phenazinyl, phenothiazinyl, furazanyl, phenoxazinyl, isochromanyl, chromanyl, imidazolidinyl, imidazolyl, pyrazolidinyl, pyrazolinyl, piperazinyl, indolyl, isindolyl, quinuclidinyl, morpholinyl, oxazolidinyl, benzotriazolyl, benzisoxazolyl, oxindolyl, benzoxazolyl, and isatinoyl.

By way of example and not limitation, carbon bonded heterocycles are bonded at position 2, 3, 4, 5, or 6 of a pyridine, position 3, 4, 5, or 6 of a pyridazine, position 2, 4, 5, or 6 of a pyrimidine, position 2, 3, 5, or 6 of a pyrazine, position 2, 3, 4, or 5 of a furan, tetrahydrofuran, thiofuran, thiophene, pyrrole or tetrahydropyrrole, position 2, 4, or 5 of an oxazole, imidazole or thiazole, position 3, 4, or 5 of an isoxazole, pyrazole, or isothiazole, position 2 or 3 of an aziridine, position 2, 3, or 4 of an azetidine, position 2, 3, 4, 5, 6, 7, or 8 of a quinoline or position 1, 3, 4, 5, 6, 7, or 8 of an isoquinoline. Still more typically, carbon bonded heterocycles include 2-pyridyl, 3-pyridyl, 4-pyridyl, 5-pyridyl, 6-pyridyl, 3-pyridazinyl, 4-pyridazinyl, 5-pyridazinyl, 6-pyridazinyl, 2-pyrimidinyl, 4-pyrimidinyl, 5-pyrimidinyl, 6-pyrimidinyl, 2-pyrazinyl, 3-pyrazinyl, 5-pyrazinyl, 6-pyrazinyl, 2-thiazolyl, 4-thiazolyl, or 5-thiazolyl.

By way of example and not limitation, nitrogen bonded heterocycles are bonded at position 1 of an aziridine, azetidine, pyrrole, pyrrolidine, 2-pyrroline, 3-pyrroline, imidazole, imidazolidine, 2-imidazoline, 3-imidazoline, pyrazole, pyrazoline, 2-pyrazoline, 3-pyrazoline, piperidine, piperazine, indole, indoline, 1H-indazole, position 2 of a isoindole, or isoindoline, position 4 of a morpholine, and position 9 of a carbazole, or β -carboline. Still more typically, nitrogen bonded heterocycles include 1-aziridyl, 1-azetedy, 1-pyrrolyl, 1-imidazolyl, 1-pyrazolyl, and 1-piperidinyl.

"Carbocycle" means a saturated or unsaturated ring having 3 to 7 carbon atoms as a monocycle or 7 to 12 carbon atoms as a bicycle. Monocyclic carbocycles have 3 to 6 ring

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atoms, still more typically 5 or 6 ring atoms. Bicyclic carbocycles have 7 to 12 ring atoms, e.g., arranged as a bicyclo [4,5], [5,5], [5,6] or [6,6] system, or 9 or 10 ring atoms arranged as a bicyclo [5,6] or [6,6] system. Examples of monocyclic carbocycles include cyclopropyl, cyclobutyl, cyclopentyl, 1-cyclopent-1-enyl, 1-cyclopent-2-enyl, 1-cyclopent-3-enyl, cyclohexyl, 1-cyclohex-1-enyl, 1-cyclohex-2-enyl, 1-cyclohex-3-enyl, cycloheptyl, and cyclooctyl.

"Linker", "Linker Unit", or "link" means a chemical moiety comprising a covalent bond or a chain of atoms that covalently attaches an antibody to a drug moiety. In various embodiments, a linker is specified as LU. Linkers include a divalent radical such as an alkylidyl, an arylidyl, a heteroarylidyl, moieties such as: $-(CR_2)_nO(CR_2)_m-$, repeating units of alkyloxy (e.g., polyethylenoxy, PEG, polymethyleneoxy) and alkylamino (e.g., polyethyleneamino, Jeffamine™); and diacid ester and amides including succinate, succinamide, diglycolate, malonate, and caproamide.

The term "chiral" refers to molecules which have the property of non-superimposability of the mirror image partner, while the term "achiral" refers to molecules which are superimposable on their mirror image partner.

The term "stereoisomers" refers to compounds which have identical chemical constitution, but differ with regard to the arrangement of the atoms or groups in space.

"Diastereomer" refers to a stereoisomer with two or more centers of chirality and whose molecules are not mirror images of one another. Diastereomers have different physical properties, e.g., melting points, boiling points, spectral properties, and reactivities. Mixtures of diastereomers may separate under high resolution analytical procedures such as electrophoresis and chromatography.

"Enantiomers" refer to two stereoisomers of a compound which are non-superimposable mirror images of one another.

Stereochemical definitions and conventions used herein generally follow S. P. Parker, Ed., *McGraw-Hill Dictionary of Chemical Terms* (1984) McGraw-Hill Book Company, New York; and Eliel, E. and Wilen, S., *Stereochemistry of Organic Compounds* (1994) John Wiley & Sons, Inc., New York. Many organic compounds exist in optically active forms, i.e., they have the ability to rotate the plane of plane-polarized light. In describing an optically active compound, the prefixes D and L, or R and S, are used to denote the absolute configuration of the molecule about its chiral center(s). The prefixes d and l or (+) and (−) are employed to designate the sign of rotation of plane-polarized light by the compound, with (−) or l meaning that the compound is levorotatory. A compound prefixed with (+) or d is dextrorotatory. For a given chemical structure, these stereoisomers are identical except that they are mirror images of one another. A specific stereoisomer may also be referred to as an enantiomer, and a mixture of such isomers is often called an enantiomeric mixture. A 50:50 mixture of enantiomers is referred to as a racemic mixture or a racemate, which may occur where there has been no stereoselection or stereospecificity in a chemical reaction or process. The terms "racemic mixture" and "racemate" refer to an equimolar mixture of two enantiomeric species, devoid of optical activity.

Examples of a "patient" include, but are not limited to, a human, rat, mouse, guinea pig, monkey, pig, goat, cow, horse, dog, cat, bird and fowl. In an exemplary embodiment, the patient is a human.

"Aryl" refers to a carbocyclic aromatic group. Examples of aryl groups include, but are not limited to, phenyl, naphthyl and anthracenyl. A carbocyclic aromatic group or

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a heterocyclic aromatic group can be unsubstituted or substituted with one or more groups including, but not limited to, $-C_1-C_8$ alkyl, $-O-(C_1-C_8 \text{ alkyl})$, -aryl, $-C(O)R'$, $-OC(O)R'$, $-C(O)OR'$, $-C(O)NH_2$, $-C(O)NHR'$, $-C(O)N(R')_2-NHC(O)R'$, $-S(O)_2R'$, $-S(O)R'$, -OH, -halogen, $-N_3$, $-NH_2$, $-NH(R')$, $-N(R')_2$ and $-CN$; wherein each R' is independently selected from H, $-C_1-C_8$ alkyl and aryl.

The term " C_1-C_8 alkyl," as used herein refers to a straight chain or branched, saturated or unsaturated hydrocarbon having from 1 to 8 carbon atoms. Representative " C_1-C_8 alkyl" groups include, but are not limited to, -methyl, -ethyl, -n-propyl, -n-butyl, -n-pentyl, -n-hexyl, -n-heptyl, -n-octyl, -n-nonyl and -n-decyl; while branched C_1-C_8 alkyls include, but are not limited to, -isopropyl, -sec-butyl, -isobutyl, -tert-butyl, -isopentyl, 2-methylbutyl, unsaturated C_1-C_8 alkyls include, but are not limited to, -vinyl, -allyl, -1-butenyl, -2-butenyl, -isobutyl, -1-pentenyl, -2-pentenyl, -3-methyl-1-butenyl, -2-methyl-2-butenyl, -2,3-dimethyl-2-butenyl, 1-hexyl, 2-hexyl, 3-hexyl, -acetylenyl, -propynyl, -1-butylnyl, -2-butylnyl, -1-pentynyl, -2-pentynyl, -3-methyl-1-butylnyl, methyl, ethyl, propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, n-pentyl, isopentyl, neopentyl, n-hexyl, isohexyl, 2-methylpentyl, 3-methylpentyl, 2,2-dimethylbutyl, 2,3-dimethylbutyl, 2,2-dimethylpentyl, 2,3-dimethylpentyl, 3,3-dimethylpentyl, 2,3,4-trimethylpentyl, 3-methylhexyl, 2,2-dimethylhexyl, 2,4-dimethylhexyl, 2,5-dimethylhexyl, 3,5-dimethylhexyl, 2,4-dimethylpentyl, 2-methylheptyl, 3-methylheptyl, n-heptyl, isohexyl, n-octyl, and isooctyl. A C_1-C_8 alkyl group can be unsubstituted or substituted with one or more groups including, but not limited to, $-C_1-C_8$ alkyl, $-O-(C_1-C_8 \text{ alkyl})$, -aryl, $-C(O)R'$, $-OC(O)R'$, $-C(O)OR'$, $-C(O)NH_2$, $-C(O)NHR'$, $-C(O)N(R')_2-NHC(O)R'$, $-SO_3R'$, $-S(O)_2R'$, $-S(O)R'$, -OH, -halogen, $-N_3$, $-NH_2$, $-NH(R')$, $-N(R')_2$ and $-CN$; where each R' is independently selected from H, $-C_1-C_8$ alkyl and aryl.

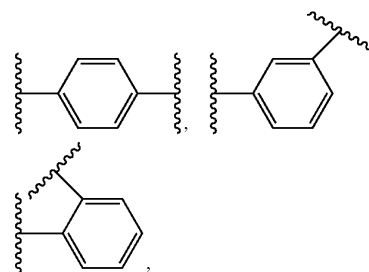
A " C_3-C_8 carbocycle" is a 3-, 4-, 5-, 6-, 7- or 8-membered saturated or unsaturated non-aromatic carbocyclic ring. Representative C_3-C_8 carbocycles include, but are not limited to, -cyclopropyl, -cyclobutyl, -cyclopentyl, -cyclopentadienyl, -cyclohexyl, -cyclohexadienyl, -1,3-cyclohexadienyl, -1,4-cyclohexadienyl, -cycloheptyl, -1,3-cycloheptadienyl, -1,3,5-cycloheptatrienyl, -cyclooctyl, and -cyclooctadienyl. A C_3-C_8 carbocycle group can be unsubstituted or substituted with one or more groups including, but not limited to, $-C_1-C_8$ alkyl, $-O-(C_1-C_8 \text{ alkyl})$, -aryl, $-C(O)R'$, $-OC(O)R'$, $-C(O)OR'$, $-C(O)NH_2$, $-C(O)NHR'$, $-C(O)N(R')_2-NHC(O)R'$, $-S(O)_2R'$, $-S(O)R'$, -OH, -halogen, $-N_3$, $-NH_2$, $-NH(R')$, $-N(R')_2$ and $-CN$; where each R' is independently selected from H, $-C_1-C_8$ alkyl and aryl.

A " C_3-C_8 carbocyclo" refers to a C_3-C_8 carbocycle group defined above wherein one of the carbocycle groups' hydrogen atoms is replaced with a bond.

A " C_1-C_{10} alkylene" is a straight chain, saturated hydrocarbon group of the formula $-(CH_2)_{1-10}-$. Examples of a C_1-C_{10} alkylene include methylene, ethylene, propylene, butylene, pentylene, hexylene, heptylene, octylene, nonylene and decalene.

An "arylene" is an aryl group which has two covalent bonds and can be in the ortho, meta, or para configurations as shown in the following structures:

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in which the phenyl group can be unsubstituted or substituted with up to four groups including, but not limited to, $-C_1-C_8$ alkyl, $-O-(C_1-C_8 \text{ alkyl})$, -aryl, $-C(O)R'$, $-OC(O)R'$, $-C(O)OR'$, $-C(O)NH_2$, $-C(O)NHR'$, $-C(O)N(R')_2-NHC(O)R'$, $-S(O)_2R'$, $-S(O)R'$, -OH, -halogen, $-N_3$, $-NH_2$, $-NH(R')$, $-N(R')_2$ and $-CN$; wherein each R' is independently selected from H, $-C_1-C_8$ alkyl and aryl.

A " C_3-C_8 heterocycle" refers to an aromatic or non-aromatic C_3-C_8 carbocycle in which one to four of the ring carbon atoms are independently replaced with a heteroatom from the group consisting of O, S and N. Representative examples of a C_3-C_8 heterocycle include, but are not limited to, benzofuranyl, benzothiophene, indolyl, benzopyrazolyl, coumarinyl, isoquinolinyl, pyrrolyl, thiophenyl, furanyl, thiazolyl, imidazolyl, pyrazolyl, triazolyl, quinolinyl, pyrimidinyl, pyridinyl, pyridonyl, pyrazinyl, pyridazinyl, isothiazolyl, isoxazolyl and tetrazolyl. A C_3-C_8 heterocycle can be unsubstituted or substituted with up to seven groups including, but not limited to, $-C_1-C_8$ alkyl, $-O-(C_1-C_8 \text{ alkyl})$, -aryl, $-C(O)R'$, $-OC(O)R'$, $-C(O)OR'$, $-C(O)NH_2$, $-C(O)NHR'$, $-C(O)N(R')_2-NHC(O)R'$, $-S(O)_2R'$, $-S(O)R'$, -OH, -halogen, $-N_3$, $-NH_2$, $-NH(R')$, $-N(R')_2$ and $-CN$; wherein each R' is independently selected from H, $-C_1-C_8$ alkyl and aryl.

" C_3-C_8 heterocyclo" refers to a C_3-C_8 heterocycle group defined above wherein one of the heterocycle group's hydrogen atoms is replaced with a bond. A C_3-C_8 heterocyclo can be unsubstituted or substituted with up to six groups including, but not limited to, $-C_1-C_8$ alkyl, $-O-(C_1-C_8 \text{ alkyl})$, -aryl, $-C(O)R'$, $-OC(O)R'$, $-C(O)OR'$, $-C(O)NH_2$, $-C(O)NHR'$, $-C(O)N(R')_2-NHC(O)R'$, $-S(O)_2R'$, $-S(O)R'$, -OH, -halogen, $-N_3$, $-NH_2$, $-NH(R')$, $-N(R')_2$ and $-CN$; wherein each R' is independently selected from H, $-C_1-C_8$ alkyl and aryl.

An "Exemplary Compound" is a Drug Compound or a Drug-Linker Compound.

An "Exemplary Conjugate" is a Drug-Ligand Conjugate having a cleavable Drug unit from the Drug-Ligand Conjugate or a Drug-Linker-Ligand Conjugate.

In some embodiments, the Exemplary Compounds and Exemplary Conjugates are in isolated or purified form. As used herein, "isolated" means separated from other components of (a) a natural source, such as a plant or animal cell or cell culture, or (b) a synthetic organic chemical reaction mixture. As used herein, "purified" means that when isolated, the isolate contains at least 95%, and in another aspect at least 98%, of Exemplary Compound or Exemplary Conjugate by weight of the isolate.

Examples of a "hydroxyl protecting group" include, but are not limited to, methoxymethyl ether, 2-methoxyethoxymethyl ether, tetrahydropyranyl ether, benzyl ether, p-methoxybenzyl ether, trimethylsilyl ether, triethylsilyl ether, triisopropyl silyl ether, t-butyldimethyl silyl ether, triphenylmethyl silyl ether, acetate ester, substituted acetate esters, pivaloate, benzoate, methanesulfonate and p-toluene-sulfonate.

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“Leaving group” refers to a functional group that can be substituted by another functional group. Such leaving groups are well known in the art, and examples include, but are not limited to, a halide (e.g., chloride, bromide, iodide), methanesulfonyl (mesyl), p-toluenesulfonyl (tosyl), trifluoromethylsulfonyl (triflate), and trifluoromethylsulfonate.

The phrase “pharmaceutically acceptable salt,” as used herein, refers to pharmaceutically acceptable organic or inorganic salts of an Exemplary Compound or Exemplary Conjugate. The Exemplary Compounds and Exemplary Conjugates contain at least one amino group, and accordingly acid addition salts can be formed with this amino group. Exemplary salts include, but are not limited to, sulfate, citrate, acetate, oxalate, chloride, bromide, iodide, nitrate, bisulfate, phosphate, acid phosphate, isonicotinate, lactate, salicylate, acid citrate, tartrate, oleate, tannate, pantothenate, bitartrate, ascorbate, succinate, maleate, gentisinate, fumarate, gluconate, glucuronate, saccharate, formate, benzoate, glutamate, methanesulfonate, ethanesulfonate, benzenesulfonate, p-toluenesulfonate, and pamoate (i.e., 1,1'-methylene-bis-(2-hydroxy-3-naphthoate)) salts. A pharmaceutically acceptable salt may involve the inclusion of another molecule such as an acetate ion, a succinate ion or other counterion. The counterion may be any organic or inorganic moiety that stabilizes the charge on the parent compound. Furthermore, a pharmaceutically acceptable salt may have more than one charged atom in its structure. Instances where multiple charged atoms are part of the pharmaceutically acceptable salt can have multiple counterions. Hence, a pharmaceutically acceptable salt can have one or more charged atoms and/or one or more counterion.

“Pharmaceutically acceptable solvate” or “solvate” refer to an association of one or more solvent molecules and a compound of the invention, e.g., an Exemplary Compound or Exemplary Conjugate. Examples of solvents that form pharmaceutically acceptable solvates include, but are not limited to, water, isopropanol, ethanol, methanol, DMSO, ethyl acetate, acetic acid, and ethanolamine.

The following abbreviations are used herein and have the indicated definitions: AE is auristatin E, Boc is N-(t-butoxycarbonyl), cit is citrulline, dap is dolaproine, DCC is 1,3-dicyclohexylcarbodiimide, DCM is dichloromethane, DEA is diethylamine, DEAD is diethylazodicarboxylate, DEPC is diethylphosphorylcanidate, DIAD is diisopropylazodicarboxylate, DIEA is N,N-diisopropylethylamine, dil is dolaisoleuine, DMAP is 4-dimethylaminopyridine, DME is ethyleneglycol dimethyl ether (or 1,2-dimethoxyethane), DMF is N,N-dimethylformamide, DMSO is dimethylsulfoxide, doe is dolaphenine, dov is N,N-dimethylvaline, DTNB is 5,5'-dithiobis(2-nitrobenzoic acid), DTPA is diethylenetriaminepentaacetic acid, DTT is dithiothreitol, EDCI is 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, EEDQ is 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline, ES-MS is electrospray mass spectrometry, EtOAc is ethyl acetate, Fmoc is N-(9-fluorenylmethoxycarbonyl), gly is glycine, HATU is O-(7-azabenzotriazol-1-yl)-N,N,N'-tetramethyluronium hexafluorophosphate, HOBt is 1-hydroxybenzotriazole, HPLC is high pressure liquid chromatography, ile is isoleucine, lys is lysine, MeCN (CH₃CN) is acetonitrile, MeOH is methanol, Mtr is 4-anisylidiphenylmethyl (or 4-methoxytrityl), nor is (1S, 2R)-(+)-norephedrine, PAB is p-aminobenzyl, PBS is phosphate-buffered saline (pH 7.4), PEG is polyethylene glycol, Ph is phenyl, Pnp is p-nitrophenyl, MC is 6-maleimidocaproyl, phe is L-phenylalanine, PyBrop is bromo tris-pyrrolidino phosphonium hexafluorophosphate, SEC is size-exclusion chromatography, Su is succinimide, TBTU is O-benzotriazol-1-yl-N,N,N,N-tetramethyluronium tetrafluoroborate, TFA is trifluoroacetic acid, TLC is thin layer chromatography, UV is ultraviolet, and val is valine.

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The following linker abbreviations are used herein and have the indicated definitions: Val Cit is a valine-citrulline, dipeptide site in protease cleavable linker; PAB is p-aminobenzylcarbonyl; (Me)vc is N-methyl-valine citrulline, where the linker peptide bond has been modified to prevent its cleavage by cathepsin B; MC(PEG)6-OH is maleimidocaproyl-polyethylene glycol; SPP is N-Succinimidyl 4-(2-pyridylthio) pentanoate; and SMCC is N-Succinimidyl 4-(N-maleimidomethyl) cyclohexane-1 carboxylate.

The terms “treat” or “treatment,” unless otherwise indicated by context, refer to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) an undesired physiological change or disorder, such as the development or spread of cancer. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (i.e., not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. “Treatment” can also mean prolonging survival as compared to expected survival if not receiving treatment. Those in need of treatment include those already with the condition or disorder as well as those prone to have the condition or disorder or those in which the condition or disorder is to be prevented.

In the context of cancer, the term “treating” includes any or all of: preventing growth of tumor cells, cancer cells, or of a tumor; preventing replication of tumor cells or cancer cells, lessening of overall tumor burden or decreasing the number of cancerous cells, and ameliorating one or more symptoms associated with the disease.

In the context of an autoimmune disease, the term “treating” includes any or all of: preventing replication of cells associated with an autoimmune disease state including, but not limited to, cells that produce an autoimmune antibody, lessening the autoimmune-antibody burden and ameliorating one or more symptoms of an autoimmune disease.

In the context of an infectious disease, the term “treating” includes any or all of: preventing the growth, multiplication or replication of the pathogen that causes the infectious disease and ameliorating one or more symptoms of an infectious disease.

The following cytotoxic drug abbreviations are used herein and have the indicated definitions: MNIAE is monomethyl auristatin E (MW 718); MMAF is N-methylvaline-valine-dolaisoleuine-dolaproine-phenylalanine (MW 731.5); MMAF-DMAEA is MMAF with DMAEA (dimethylaminoethylamine) in an amide linkage to the C-terminal phenylalanine (MW 801.5); MMAF-TEG is MMAF with tetraethylene glycol esterified to the phenylalanine; MMAF-NtBu is N-t-butyl, attached as an amide to C-terminus of MMAF; AEVB is auristatin E valeryl benzylhydrazide, acid labile linker through the C-terminus of AE (MW 732); and AFP is Monoamide of p-phenylene diamine with C-terminal Phenylalanine of Auristatin F (MW 732).

9.2 The Compounds of the Invention

9.2.1 The Compounds of Formula (Ia)

In one aspect, the invention provides Drug-Linker-Ligand Conjugates having Formula Ia:



or a pharmaceutically acceptable salt or solvate thereof wherein,

L- is a Ligand unit;

-A_x-W_y-Y_z- is a Linker unit (LU), wherein the Linker unit includes:

-A- is a Stretcher unit,

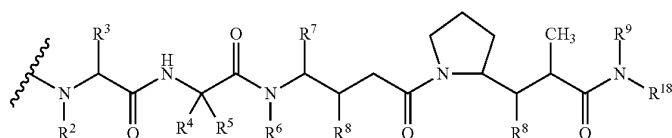
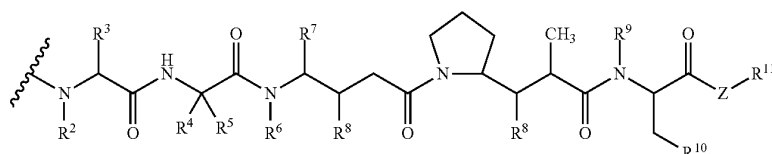
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a is 0 or 1,
 each —W— is independently an Amino Acid unit,
 w is an integer ranging from 0 to 12,
 —Y— is a Spacer unit, and
 y is 0, 1 or 2;
 p ranges from 1 to about 20; and
 -D is a Drug unit having the Formulas D_E and D_F:

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m is an integer ranging from 1-1000;
 R¹³ is C₂-C₈ alkyl;
 R¹⁴ is H or C₁-C₈ alkyl;
 each occurrence of R¹⁵ is independently H, COOH,
 —(CH₂)_n—N(R¹⁶)₂, —(CH₂)_n—SO₃H, or
 —(CH₂)_n—SO₃—C₁-C₈ alkyl;

D_ED_F

wherein, independently at each location:

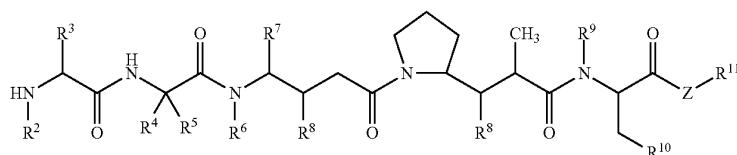
R² is selected from H and C₁-C₈ alkyl;
 R³ is selected from H, C₁-C₈ alkyl, C₃-C₈ carbocycle,
 aryl, C₁-C₈ alkyl-aryl, C₁-C₈ alkyl-(C₃-C₈ carbocycle),
 C₃-C₈ heterocycle and C₁-C₈ alkyl-(C₃-C₈ hetero-
 cycle);
 R⁴ is selected from H, C₁-C₈ alkyl, C₃-C₈ carbocycle,
 aryl, C₁-C₈ alkyl-aryl, C₁-C₈ alkyl-(C₃-C₈ carbocycle),
 C₃-C₈ heterocycle and C₁-C₈ alkyl-(C₃-C₈ hetero-
 cycle);

each occurrence of R¹⁶ is independently H, C₁-C₈ alkyl,
 or —(CH₂)_n—COOH;

R¹⁸ is selected from —C(R⁸)₂—C(R⁸)₂-aryl, —C(R⁸)₂—
 C(R⁸)₂—(C₃-C₈ heterocycle), and —C(R⁸)₂—
 C(R⁸)₂—(C₃-C₈ carbocycle); and

n is an integer ranging from 0 to 6.

In another embodiment, the present invention provides
 Drug Compounds having the Formula Ib:



Ib

Ib

R⁵ is selected from H and methyl;

or R⁴ and R⁵ jointly form a carbocyclic ring and have the
 formula —(CR^aR^b)_n— wherein R^a and R^b are indepen-
 dently selected from H, C₁-C₈ alkyl and C₃-C₈ carbo-
 cycle and n is selected from 2, 3, 4, 5 and 6;

R⁶ is selected from H and C₁-C₈ alkyl;

R⁷ is selected from H, C₁-C₈ alkyl, C₃-C₈ carbocycle,
 aryl, C₁-C₈ alkyl-aryl, C₁-C₈ alkyl-(C₃-C₈ carbocycle),
 C₃-C₈ heterocycle and C₁-C₈ alkyl-(C₃-C₈ hetero-
 cycle);

each R⁸ is independently selected from H, OH, C₁-C₈
 alkyl, C₃-C₈ carbocycle and O—(C₁-C₈ alkyl);

R⁹ is selected from H and C₁-C₈ alkyl;

R¹⁰ is selected from aryl or C₃-C₈ heterocycle;

Z is O, S, NH, or NR¹², wherein R¹² is C₁-C₈ alkyl;

R¹¹ is selected from H, C₁-C₂₀ alkyl, aryl, C₃-C₈ hetero-
 cycle, —(R¹³O)_m—R¹⁴, or —(R¹³O)_m—CH(R¹⁵)₂;

or pharmaceutically acceptable salts or solvates thereof,
 wherein:

R² is selected from hydrogen and —C₁-C₈ alkyl;

R³ is selected from hydrogen, —C₁-C₈ alkyl, —C₃-C₈
 carbocycle, aryl, —C₁-C₈ alkyl-aryl, —C₁-C₈ alkyl-
 (C₃-C₈ carbocycle), —C₃-C₈ heterocycle and —C₁-C₈
 alkyl-(C₃-C₈ heterocycle);

R⁴ is selected from hydrogen, —C₁-C₈ alkyl, —C₃-C₈
 carbocycle, -aryl, —C₁-C₈ alkyl-aryl, —C₁-C₈ alkyl-
 (C₃-C₈ carbocycle), —C₃-C₈ heterocycle and —C₁-C₈
 alkyl-(C₃-C₈ heterocycle) wherein R⁵ is selected from
 —H and -methyl; or R⁴ and R⁵ jointly, have the formula
 —(CR^aR^b)_n— wherein R^a and R^b are independently
 selected from —H, —C₁-C₈ alkyl and —C₃-C₈ carbo-
 cycle and n is selected from 2, 3, 4, 5 and 6, and form
 a ring with the carbon atom to which they are attached;

R⁶ is selected from H and —C₁-C₈ alkyl;

R⁷ is selected from H, —C₁-C₈ alkyl, —C₃-C₈ carbo-
 cycle, aryl, —C₁-C₈ alkyl-aryl, —C₁-C₈ alkyl-(C₃-C₈
 carbocycle), —C₃-C₈ heterocycle and —C₁-C₈ alkyl-
 (C₃-C₈ heterocycle);

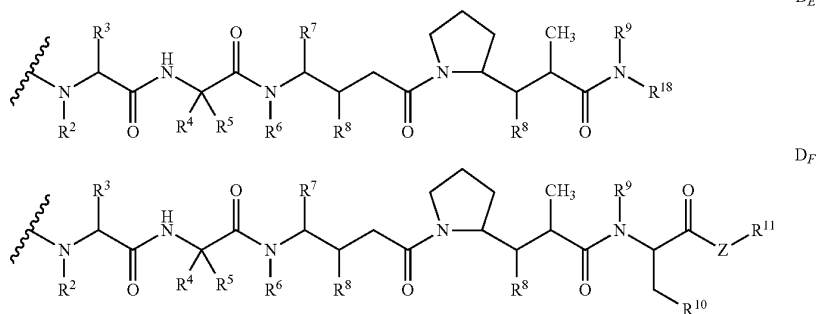
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each R⁸ is independently selected from H, —OH, —C₁-C₈ alkyl, —C₃-C₈ carbocycle and —O—(C₁-C₈ alkyl);
 R⁹ is selected from H and —C₁-C₈ alkyl;
 R¹⁰ is selected from aryl group or —C₃-C₈ heterocycle;
 Z is —O—, —S—, —NH—, or —NR¹²—, wherein R¹² is C₁-C₈ alkyl;
 R¹¹ is selected from H, C₁-C₂₀ alkyl, aryl, —C₃-C₈ heterocycle, —(R¹³O)_m—R¹⁴, or —(R¹³O)_m—CH(R¹⁵)₂;
 m is an integer ranging from 1-1000;
 R¹³ is —C₂-C₈ alkyl;
 R¹⁴ is H or —C₁-C₈ alkyl;
 each occurrence of R¹⁵ is independently H, —COOH, —(CH₂)_n—N(R¹⁶)₂, —(CH₂)_n—SO₃H, or —(CH₂)_n—SO₃—C₁-C₈ alkyl;
 each occurrence of R¹⁶ is independently H, —C₁-C₈ alkyl, or —(CH₂)_n—COOH; and
 n is an integer ranging from 0 to 6.
 In yet another embodiment, the invention provides Drug-Linker-Ligand Conjugates having the Formula Ia':

$$Ab-(A_x-W_w-Y_p-D_p) \quad \text{Formula Ia'}$$

or pharmaceutically acceptable salts or solvates thereof, wherein:
 Ab is an antibody,
 A is a Stretcher unit,
 a is 0 or 1,
 each W is independently an Amino Acid unit,
 w is an integer ranging from 0 to 12,
 Y is a Spacer unit, and
 y is 0, 1 or 2,
 p ranges from 1 to about 20, and
 D is a Drug moiety selected from Formulas D_E and D_F:



wherein, independently at each location:

R² is selected from H and C₁-C₈ alkyl;
 R³ is selected from H, C₁-C₈ alkyl, C₃-C₈ carbocycle, aryl, C₁-C₈ alkyl-aryl, C₁-C₈ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₈ alkyl-(C₃-C₈ heterocycle);
 R⁴ is selected from H, C₁-C₈ alkyl, C₃-C₈ carbocycle, aryl, C₁-C₈ alkyl-aryl, C₁-C₈ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₈ alkyl-(C₃-C₈ heterocycle);
 R⁵ is selected from H and methyl;
 or R⁴ and R⁵ jointly form a carbocyclic ring and have the formula —(CR^aR^b)_n— wherein R^a and R^b are independently selected from H, C₁-C₈ alkyl and C₃-C₈ carbocycle and n is selected from 2, 3, 4, 5 and 6;
 R⁶ is selected from H and C₁-C₈ alkyl;

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R⁷ is selected from H, C₁-C₈ alkyl, C₃-C₈ carbocycle, aryl, C₁-C₈ alkyl-aryl, C₁-C₈ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₈ alkyl-(C₃-C₈ heterocycle);
 each R⁸ is independently selected from H, OH, C₁-C₈ alkyl, C₃-C₈ carbocycle and O—(C₁-C₈ alkyl);
 R⁹ is selected from H and C₁-C₈ alkyl;
 R¹⁰ is selected from aryl or C₃-C₈ heterocycle;
 Z is O, S, NH, or NR¹², wherein R¹² is C₁-C₈ alkyl;
 R¹¹ is selected from H, C₁-C₂₀ alkyl, aryl, C₃-C₈ heterocycle, —(R¹³O)_m—R¹⁴, or —(R¹³O)_m—CH(R¹⁵)₂;
 m is an integer ranging from 1-1000;
 R¹³ is C₂-C₈ alkyl;
 R¹⁴ is H or C₁-C₈ alkyl;
 each occurrence of R¹⁵ is independently H, COOH, —(CH₂)_n—N(R¹⁶)₂, —(CH₂)_n—SO₃H, or —(CH₂)_n—SO₃—C₁-C₈ alkyl;
 each occurrence of R¹⁶ is independently H, C₁-C₈ alkyl, or —(CH₂)_n—COOH;
 R¹⁸ is selected from —C(R⁸)₂—C(R⁸)₂—aryl, —C(R⁸)₂—C(R⁸)₂—(C₃-C₈ heterocycle), and —C(R⁸)₂—C(R⁸)₂—(C₃-C₈ carbocycle); and
 n is an integer ranging from 0 to 6.
 Ab is any antibody covalently attached to one or more drug units. Ab includes an antibody which binds to CD30, CD40, CD70, Lewis Y antigen. In another embodiment, Ab does not include an antibody which binds to an ErbB receptor or to one or more of receptors (1)-(35):
 (1) BMPR1B (bone morphogenetic protein receptor-type IB, Genbank accession no. NM_001203);
 (2) E16 (LAT1, SLC7A5, Genbank accession no. NM_003486);

(3) STEAP1 (six transmembrane epithelial antigen of prostate, Genbank accession no. NM_012449);
 (4) 0772P (CA125, MUC16, Genbank accession no. AF361486);
 (5) MPF (MPF, MSLN, SMR, megakaryocyte potentiating factor, mesothelin, Genbank accession no. NM_005823);
 (6) Napi3b (NAPI-3B, NPTIIB, SLC34A2, solute carrier family 34 (sodium phosphate), member 2, type II sodium-dependent phosphate transporter 3b, Genbank accession no. NM_006424);
 (7) Sema 5b (FLJ10372, KIAA1445, Mm.42015, SEMASB, SEMAG, Semaphorin 5b Hlog, sema domain, seven thrombospondin repeats (type 1 and type 1-like), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 5B, Genbank accession no. AB040878);

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- (8) PSCA hlg (2700050C12Rik, C₅₃₀₀₀₈₀₁₆Rik, RIKEN cDNA 2700050C12, RIKEN cDNA 2700050C12 gene, Genbank accession no. AY358628);
- (9) ETBR (Endothelin type B receptor, Genbank accession no. AY275463);
- (10) MSG783 (RNF124, hypothetical protein FLJ20315, Genbank accession no. NM_017763);
- (11) STEAP2 (HGNC_8639, IPCA-1, PCANAP1, STAMP1, STEAP2, STMP, prostate cancer associated gene 1, prostate cancer associated protein 1, six transmembrane epithelial antigen of prostate 2, six transmembrane prostate protein, Genbank accession no. AF455138);
- (12) TrpM4 (BR22450, FLJ20041, TRPM4, TRPM4B, transient receptor potential cation channel, subfamily M, member 4, Genbank accession no. NM_017636);
- (13) CRIPTO (CR, CR1, CRGF, CRIPTO, TDGF1, teratocarcinoma-derived growth factor, Genbank accession no. NP_003203 or NM_003212);
- (14) CD21 (CR₂ (Complement receptor 2) or C3DR (C3d/Epstein Barr virus receptor) or Hs.73792, Genbank accession no. M26004);
- (15) CD79b (IGb (immunoglobulin-associated beta), B29, Genbank accession no. NM_000626);
- (16) FcRH2 (IFGP4, IRTA4, SPAP1A (SH2 domain containing phosphatase anchor protein 1a), SPAP1B, SPAP1C, Genbank accession no. NM_030764);
- (17) HER2 (Genbank accession no. M11730);
- (18) NCA (Genbank accession no. M18728);
- (19) MDP (Genbank accession no. BC017023);
- (20) IL20Rα (Genbank accession no. AF184971);
- (21) Brevican (Genbank accession no. AF229053);
- (22) Ephb2R (Genbank accession no. NM_004442);
- (23) ASLG659 (Genbank accession no. AX092328);
- (24) PSCA (Genbank accession no. AJ297436);
- (25) GEDA (Genbank accession no. AY260763);
- (26) BAFF-R (Genbank accession no. NP_443177.1);
- (27) CD22 (Genbank accession no. NP_001762.1);
- (28) CD79a (CD79A, CD79a, immunoglobulin-associated alpha, a B cell-specific protein that covalently interacts with Ig beta (CD79B) and forms a complex on the surface with Ig M molecules, transduces a signal involved in B-cell differentiation, Genbank accession No. NP_001774.1);
- (29) CXCR5 (Burkitt's lymphoma receptor 1, a G protein-coupled receptor that is activated by the CXCL13 chemokine, functions in lymphocyte migration and humoral defense, plays a role in HIV-2 infection and perhaps development of AIDS, lymphoma, myeloma, and leukemia, Genbank accession No. NP_001707.1);
- (30) HLA-DOB (Beta subunit of MHC class II molecule (Ia antigen) that binds peptides and presents them to CD4+T lymphocytes, Genbank accession No. NP_002111.1);

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- (31) P2X5 (Purinergic receptor P2X ligand-gated ion channel 5, an ion channel gated by extracellular ATP, may be involved in synaptic transmission and neurogenesis, deficiency may contribute to the pathophysiology of idiopathic detrusor instability, Genbank accession No. NP_002552.2);
- (32) CD72 (B-cell differentiation antigen CD72, Lyb-2, Genbank accession No. NP_001773.1);
- (33) LY64 (Lymphocyte antigen 64 (RP105), type I membrane protein of the leucine rich repeat (LRR) family, regulates B-cell activation and apoptosis, loss of function is associated with increased disease activity in patients with systemic lupus erythematosus, Genbank accession No. NP_005573.1);
- (34) FCRH1 (Fc receptor-like protein 1, a putative receptor for the immunoglobulin Fc domain that contains C₂ type Ig-like and ITAM domains, may have a role in B-lymphocyte differentiation, Genbank accession No. NP_443170.1); and/or
- (35) IRTA2 (Immunoglobulin superfamily receptor translocation associated 2, a putative immunoreceptor with possible roles in B cell development and lymphomagenesis; deregulation of the gene by translocation occurs in some B cell malignancies, Genbank accession No. NP_112571.1).

In one embodiment -Ww- is -Val-Cit-.

In another embodiment, R³, R⁴ and R⁷ are independently isopropyl or sec-butyl and R⁵ is —H. In an exemplary embodiment, R³ and R⁴ are each isopropyl, R⁵ is —H, and R⁷ is sec-butyl. In yet another embodiment, R² and R⁶ are each methyl, and R⁹ is —H.

In still another embodiment, each occurrence of R⁸ is —OCH₃.

In an exemplary embodiment, R³ and R⁴ are each isopropyl, R² and R⁶ are each methyl, R⁵ is —H, R⁷ is sec-butyl, each occurrence of R⁸ is —OCH₃, and R⁹ is —H.

In one embodiment, Z is —O— or —NH—.

In one embodiment, R¹⁰ is aryl In an exemplary embodiment, R¹⁰ is -phenyl.

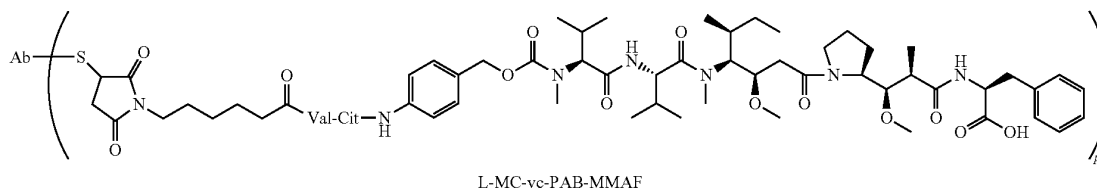
In an exemplary embodiment, when Z is —O—, R¹¹ is —H, methyl or t-butyl.

In one embodiment, when Z is —NH, R¹¹ is —CH(R¹⁵)₂, wherein R¹⁵ is —(CH₂)_n—N(R¹⁶)₂, and R¹⁶ is —C₁—C₈ alkyl or —(CH₂)_n—COOH.

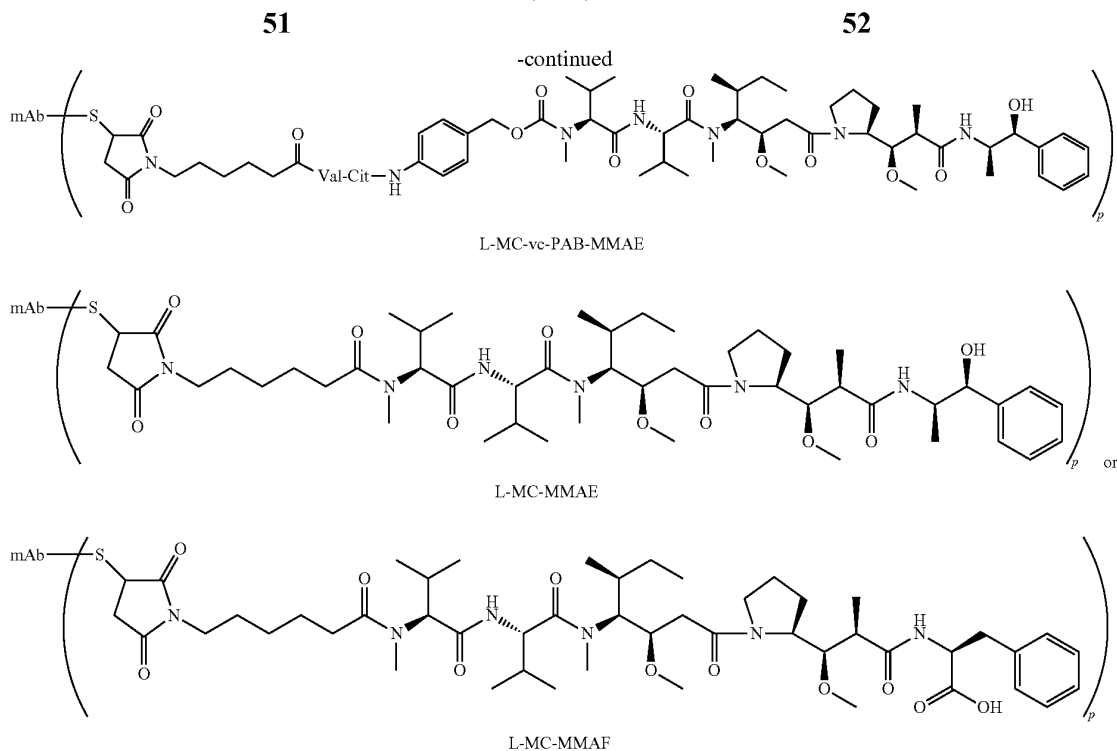
In another embodiment, when Z is —NH, R¹¹ is —CH(R¹⁵)₂, wherein R¹⁵ is —(CH₂)_n—SO₃H.

In one aspect, Ab is cAC10, cBR96, cS2C₆, c1F6, c2F2, hAC10, hBR96, hS2C₆, h1F6, and h2F2.

Exemplary embodiments of Formula Ia have the following structures:



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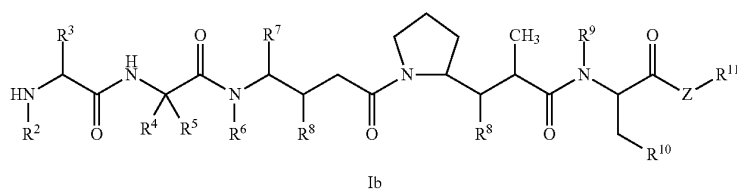


wherein L is an antibody, Val is valine, and Cit is citrulline.

The drug loading is represented by p, the average number of drug molecules per antibody in a molecule (e.g., of Formula Ia, Ia' and Ic). Drug loading may range from 1 to 20 drugs (D) per Ligand (e.g., Ab or mAb). Compositions of Formula Ia and Formula Ia' include collections of antibodies conjugated with a range of drugs, from 1 to 20. The average number of drugs per antibody in preparation of conjugation reactions may be characterized by conventional means such as mass spectroscopy, ELISA assay, and HPLC. The quantitative distribution of Ligand-Drug-Conjugates in terms of p may also be determined. In some instances, separation, purification, and characterization of homogeneous Ligand-Drug-conjugates where p is a certain value from Ligand-Drug-Conjugates with other drug loadings may be achieved by means such as reverse phase HPLC or electrophoresis.

9.2.2 The Drug Compounds of Formula (Ib)

In another aspect, the present invention provides Drug Compounds having the Formula (Ib):



or a pharmaceutically acceptable salt or solvate thereof, wherein:

R² is selected from -hydrogen and -C₁-C₈ alkyl;

R³ is selected from -hydrogen, -C₁-C₈ alkyl, -C₃-C₈ carbocycle, aryl, -C₁-C₈ alkyl-aryl, -C₁-C₈ alkyl-(C₃-C₈ carbocycle), -C₃-C₈ heterocycle and -C₁-C₈ alkyl-(C₃-C₈ heterocycle);

R⁴ is selected from -hydrogen, -C₁-C₈ alkyl, -C₃-C₈ carbocycle, -aryl, -C₁-C₈ alkyl-aryl, -C₁-C₈ alkyl-(C₃-C₈ carbocycle), -C₃-C₈ heterocycle and -C₁-C₈ alkyl-(C₃-C₈ heterocycle) wherein R⁵ is selected from -H and -methyl; or R⁴ and R⁵ jointly, have the formula -(CR^aR^b)_n wherein R^a and R^b are independently selected from -H, -C₁-C₈ alkyl and -C₃-C₈ carbocycle and n is selected from 2, 3, 4, 5 and 6, and form a ring with the carbon atom to which they are attached;

R⁶ is selected from -H and -C₁-C₈ alkyl;

R⁷ is selected from -H, -C₁-C₈ alkyl, -C₃-C₈ carbocycle, aryl, -C₁-C₈ alkyl-aryl, -C₁-C₈ alkyl-(C₃-C₈ carbocycle), -C₃-C₈ heterocycle and -C₁-C₈ alkyl-(C₃-C₈ heterocycle);

each R⁸ is independently selected from -H, -OH, -C₁-C₈ alkyl, -C₃-C₈ carbocycle and -O-(C₁-C₈ alkyl);

R⁹ is selected from -H and -C₁-C₈ alkyl;

R¹⁰ is selected from aryl group or -C₃-C₈ heterocycle;

Ib

Z is -O-, -S-, -NH-, or -NR¹²-, wherein R¹² is C₁-C₈ alkyl;

R¹¹ is selected from -H, C₁-C₂₀ alkyl, aryl, -C₃-C₈ heterocycle, -(R¹³O)_m-R¹⁴, or -(R¹³O)_m-CH(R¹⁵)₂;

m is an integer ranging from 1-1000;

R¹³ is -C₂-C₈ alkyl;

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R^{14} is $-H$ or $-C_1-C_8$ alkyl;
 each occurrence of R^{15} is independently $-H$, $-COOH$,
 $-(CH_2)_n-N(R^{16})_2$, $-(CH_2)_n-SO_3H$, or
 $-(CH_2)_n-SO_3-C_1-C_8$ alkyl;
 each occurrence of R^{16} is independently $-H$, $-C_1-C_8$ 5
 alkyl, or $-(CH_2)_n-COOH$; and
 n is an integer ranging from 0 to 6.

In one embodiment, R^3 , R^4 and R^7 are independently
 isopropyl or sec-butyl and R^5 is $-H$. In an exemplary
 embodiment, R^3 and R^4 are each isopropyl, R^5 is $-H$, and 10
 R^7 is sec-butyl.

In another embodiment, R^2 and R^6 are each methyl, and
 R^9 is $-H$.

In still another embodiment, each occurrence of R^8 is
 $-OCH_3$.

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In an exemplary embodiment, R^3 and R^4 are each isopro-
 pyl, R^2 and R^6 are each methyl, R^5 is $-H$, R^7 is sec-butyl,
 each occurrence of R^8 is $-OCH_3$, and R^9 is $-H$.

In one embodiment, Z is $-O-$ or $-NH-$.

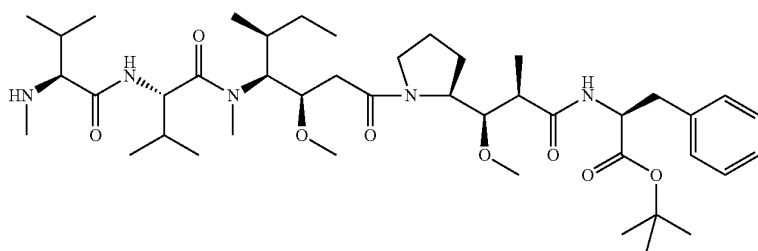
In one embodiment, R^{10} is aryl

In an exemplary embodiment, R^{10} is -phenyl.

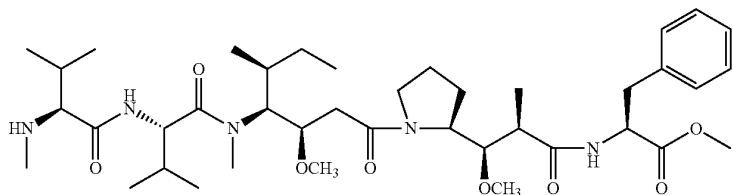
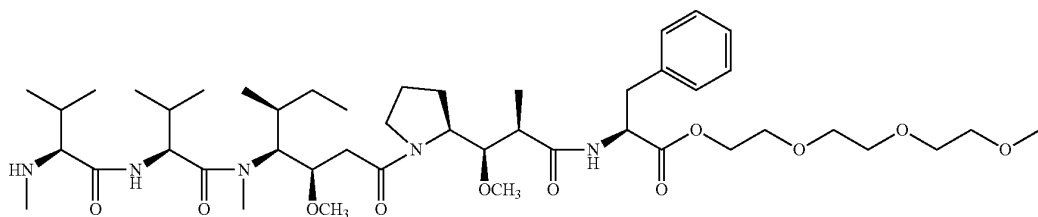
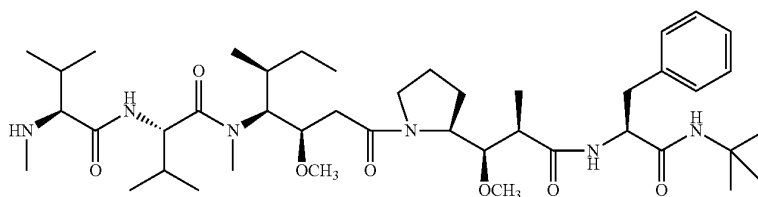
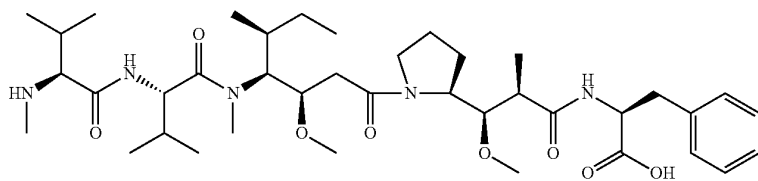
In an exemplary embodiment, when Z is $-O-$, R^{11} is
 $-H$, methyl or t-butyl.

In one embodiment, when Z is $-NH$, R^{11} is $-CH(R^{15})_2$,
 wherein R^{15} is $-(CH_2)_n-N(R^{16})_2$, and R^{16} is $-C_1-C_8$
 alkyl or $-(CH_2)_n-COOH$.

In another embodiment, when Z is $-NH$, R^{11} is $-CH$
 $(R^{15})_2$, wherein R^{15} is $-(CH_2)_n-SO_3H$. Illustrative Com-
 pounds of Formula (Ib), each of which may be used as drug
 moieties (D) in ADC, include compounds having the fol-
 lowing structures:



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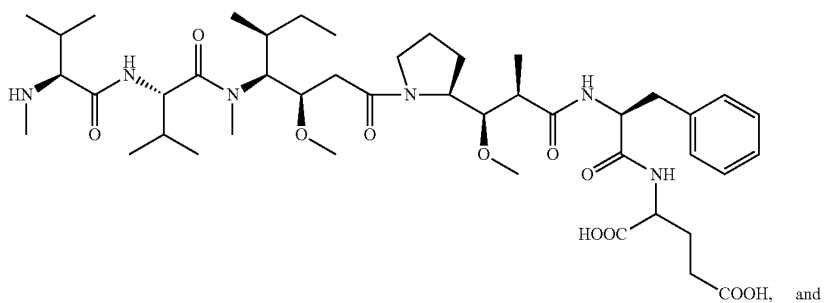
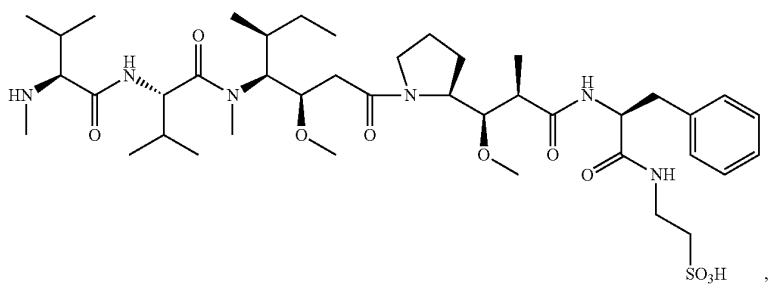
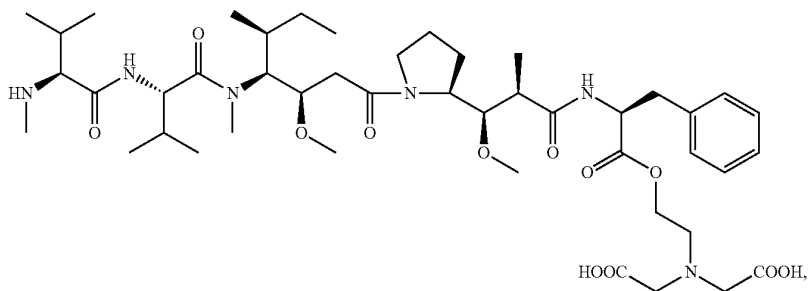
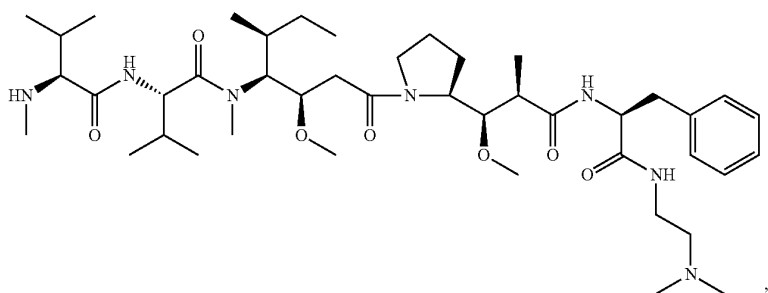


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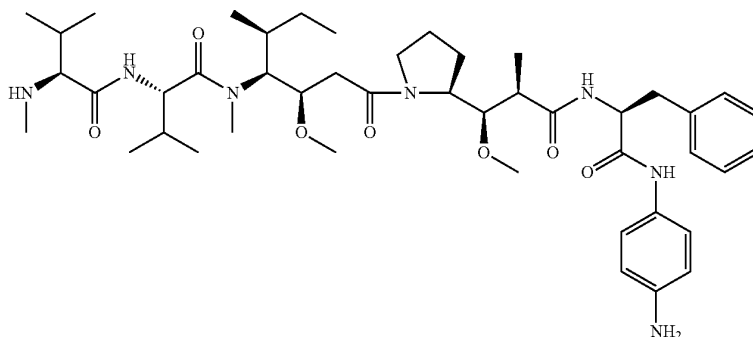
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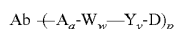
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and pharmaceutically acceptable salts or solvates thereof.
9.2.3 The Compounds of Formula (Ic)

In another aspect, the invention provides antibody-drug
conjugate compounds (ADC) having Formula Ic:



Ic

comprising an antibody covalently attached to one or more
drug units (moieties). The antibody-drug conjugate compounds
include pharmaceutically acceptable salts or solvates
thereof. Formula Ic compounds are defined wherein:

Ab is an antibody which binds to one or more tumor-
associated antigen receptors (1)-(35):

- (1) BMPR1B (bone morphogenetic protein receptor-type
IB, Genbank accession no. NM_001203);
- (2) E16 (LAT1, SLC7A5, Genbank accession no.
NM_003486);
- (3) STEAP1 (six transmembrane epithelial antigen of
prostate, Genbank accession no. NM_012449);
- (4) 0772P (CA125, MUC16, Genbank accession no.
AF361486);
- (5) MPF (MPF, MSLN, SMR, megakaryocyte potenti-
ating factor, mesothelin, Genbank accession no. 40
NM_005823);
- (6) Napi3b (NAPI-3B, NPTIIB, SLC34A2, solute carrier
family 34 (sodium phosphate), member 2, type II
sodium-dependent phosphate transporter 3b, Genbank
accession no. NM_006424);
- (7) Sema 5b (F1110372, KIAA1445, Mm.42015,
SEMA5B, SEMAG, Semaphorin 5b Hlog, sema
domain, seven thrombospondin repeats (type 1 and
type 1-like), transmembrane domain (TM) and short
cytoplasmic domain, (semaphorin) 5B, Genbank acces- 45
sion no. AB040878);
- (8) PSCA hlg (2700050C12Rik, C₅₃₀₀₀₈O16Rik, RIKEN
cDNA 2700050C12, RIKEN cDNA 2700050C12 gene,
Genbank accession no. AY358628);
- (9) ETBR (Endothelin type B receptor, Genbank acces- 55
sion no. AY275463);
- (10) MSG783 (RNF124, hypothetical protein F1120315,
Genbank accession no. NM_017763);
- (11) STEAP2 (HGNC_8639, IPCA-1, PCANAP1,
STAMP1, STEAP2, STMP, prostate cancer associated
gene 1, prostate cancer associated protein 1, six trans-
membrane epithelial antigen of prostate 2, six trans-
membrane prostate protein, Genbank accession no.
AF455138);
- (12) TrpM4 (BR22450, F1120041, TRPM4, TRPM4B, 65
transient receptor potential cation channel, subfamily
M, member 4, Genbank accession no. NM_017636);

- (13) CRIPTO (CR, CR1, CRGF, CRIPTO, TDGF1,
teratocarcinoma-derived growth factor, Genbank
accession no. NP_003203 or NM_003212);
- (14) CD21 (CR₂ (Complement receptor 2) or C3DR
(C3d/Epstein Barr virus receptor) or Hs.73792 Gen-
bank accession no. M26004);
- (15) CD79b (CD79B, CD79β, IgB (immunoglobulin-
associated beta), B29, Genbank accession no.
NM_000626);
- (16) FcRH2 (IFGP4, IRTA4, SPAP1A (SH2 domain con-
taining phosphatase anchor protein 1a), SPAP1B,
SPAP1C, Genbank accession no. NM_030764);
- (17) HER2 (Genbank accession no. M11730);
- (18) NCA (Genbank accession no. M18728);
- (19) MDP (Genbank accession no. BC017023);
- (20) IL20Rα (Genbank accession no. AF184971);
- (21) Brevican (Genbank accession no. AF229053);
- (22) Ephb2R (Genbank accession no. NM_004442);
- (23) ASLG659 (Genbank accession no. AX092328);
- (24) PSCA (Genbank accession no. AJ297436);
- (25) GEDA (Genbank accession no. AY260763);
- (26) BAFF-R (B cell-activating factor receptor, BLyS
receptor 3, BR3, NP_443177.1);
- (27) CD22 (B-cell receptor CD22-B isoform,
NP_001762.1);
- (28) CD79a (CD79A, CD79a, immunoglobulin-associ-
ated alpha, a B cell-specific protein that covalently
interacts with Ig beta (CD79B) and forms a complex on
the surface with Ig M molecules, transduces a signal
involved in B-cell differentiation, Genbank accession
No. NP_001774.1);
- (29) CXCR5 (Burkitt's lymphoma receptor 1, a G pro-
tein-coupled receptor that is activated by the CXCL13
chemokine, functions in lymphocyte migration and
humoral defense, plays a role in HIV-2 infection and
perhaps development of AIDS, lymphoma, myeloma,
and leukemia, Genbank accession No. NP_001707.1);
- (30) HLA-DOB (Beta subunit of MHC class II molecule
(Ia antigen) that binds peptides and presents them to
CD4+T lymphocytes, Genbank accession No.
NP_002111.1);
- (31) P2X5 (Purinergic receptor P2X ligand-gated ion
channel 5, an ion channel gated by extracellular ATP,
may be involved in synaptic transmission and neuro-
genesis, deficiency may contribute to the pathophysi-
ology of idiopathic detrusor instability, Genbank acces-
sion No. NP_002552.2);
- (32) CD72 (B-cell differentiation antigen CD72, Lyb-2,
Genbank accession No. NP_001773.1);

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(33) LY64 (Lymphocyte antigen 64 (RP105), type I membrane protein of the leucine rich repeat (LRR) family, regulates B-cell activation and apoptosis, loss of function is associated with increased disease activity in patients with systemic lupus erythematosus, Genbank accession No. NP_005573.1);

(34) FCRH1 (Fc receptor-like protein 1, a putative receptor for the immunoglobulin Fc domain that contains C₂ type Ig-like and ITAM domains, may have a role in B-lymphocyte differentiation, Genbank accession No. NP_443170.1); and

(35) IRTA2 (Immunoglobulin superfamily receptor translocation associated 2, a putative immunoreceptor with possible roles in B cell development and lymphomagenesis; deregulation of the gene by translocation occurs in some B cell malignancies, Genbank accession No. NP_112571.1).

A is a Stretcher unit,

a is 0 or 1,

each W is independently an Amino Acid unit,

w is an integer ranging from 0 to 12,

Y is a Spacer unit, and

y is 0, 1 or 2,

p ranges from 1 to about 8, and

D is a Drug moiety selected from Formulas D_E and D_F:

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R⁷ is selected from H, C₁-C₈ alkyl, C₃-C₈ carbocycle, aryl, C₁-C₈ alkyl-aryl, C₁-C₈ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₈ alkyl-(C₃-C₈ heterocycle);

each R⁸ is independently selected from H, OH, C₁-C₈ alkyl, C₃-C₈ carbocycle and O-(C₁-C₈ alkyl);

R⁹ is selected from H and C₁-C₈ alkyl;

R¹⁰ is selected from aryl or C₃-C₈ heterocycle;

Z is O, S, NH, or NR¹², wherein R¹² is C₁-C₈ alkyl;

R¹¹ is selected from H, C₁-C₂₀ alkyl, aryl, C₃-C₈ heterocycle, -(R¹³O)_m-R¹⁴, or -(R¹³O)_m-CH(R¹⁵)₂;

m is an integer ranging from 1-1000;

R¹³ is C₂-C₈ alkyl;

R¹⁴ is H or C₁-C₈ alkyl;

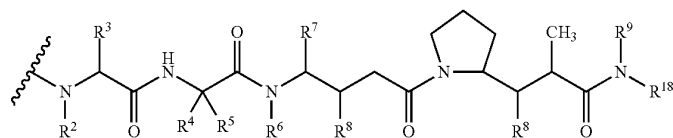
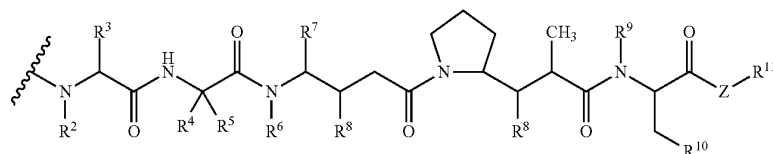
each occurrence of R¹⁵ is independently H, COOH, -(CH₂)_n-N(R¹⁶)₂, -(CH₂)_n-SO₃H, or -(CH₂)_n-SO₃-C₁-C₈ alkyl;

each occurrence of R¹⁶ is independently H, C₁-C₈ alkyl, or -(CH₂)_n-COOH; R¹⁸ is selected from

-C(R⁸)₂-C(R⁸)₂-aryl, -C(R⁸)₂-C(R⁸)₂-(C₃-C₈ heterocycle), and -C(R⁸)₂-C(R⁸)₂-(C₃-C₈ carbocycle); and

n is an integer ranging from 0 to 6.

In one embodiment -Ww- is -Val-Cit-.

D_ED_F

wherein the wavy line of D_E and D_F indicates the covalent attachment site to A, W, or Y, and independently at each location:

R² is selected from H and C₁-C₈ alkyl;

R³ is selected from H, C₁-C₈ alkyl, C₃-C₈ carbocycle, aryl, C₁-C₈ alkyl-aryl, C₁-C₈ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₈ alkyl-(C₃-C₈ heterocycle);

R⁴ is selected from H, C₁-C₈ alkyl, C₃-C₈ carbocycle, aryl, C₁-C₈ alkyl-aryl, C₁-C₈ alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and C₁-C₈ alkyl-(C₃-C₈ heterocycle);

R⁵ is selected from H and methyl;

or R⁴ and R⁵ jointly form a carbocyclic ring and have the formula -(CR^aR^b)_n- wherein R^a and R^b are independently selected from H, C₁-C₈ alkyl and C₃-C₈ carbocycle and n is selected from 2, 3, 4, 5 and 6;

R⁶ is selected from H and C₁-C₈ alkyl;

In another embodiment, R³, R⁴ and R⁷ are independently isopropyl or sec-butyl and R⁵ is -H. In an exemplary embodiment, R³ and R⁴ are each isopropyl, R⁵ is -H, and R⁷ is sec-butyl.

In yet another embodiment, R² and R⁶ are each methyl, and R⁹ is -H.

In still another embodiment, each occurrence of R⁸ is -OCH₃.

In an exemplary embodiment, R³ and R⁴ are each isopropyl, R² and R⁶ are each methyl, R⁵ is -H, R⁷ is sec-butyl, each occurrence of R⁸ is -OCH₃, and R⁹ is -H.

In one embodiment, Z is -O- or -NH-.

In one embodiment, R¹⁰ is aryl.

In an exemplary embodiment, R¹⁰ is -phenyl.

In an exemplary embodiment, when Z is -O-, R¹¹ is -H, methyl or t-butyl.

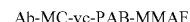
In one embodiment, when Z is -NH, R¹¹ is -CH(R¹⁵)₂, wherein R¹⁵ is -(CH₂)_n-N(R¹⁶)₂, and R¹⁶ is -C₁-C₈ alkyl or -(CH₂)_n-COOH.

In another embodiment, when Z is -NH, R¹¹ is -CH(R¹⁵)₂, wherein R¹⁵ is -(CH₂)_n-SO₃H.

Exemplary embodiments of Formula 1c ADC have the following structures:

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Ab-MC-vc-PAB-MMAE

Ah-MC-MMAF

Ab-MC-MMAF

It is to be understood that where more than one nucleophilic group reacts with a drug-linker intermediate, or linker reagent followed by drug moiety reagent, then the resulting product is a mixture of ADC compounds with a distribution of one or more drug moieties attached to an antibody. The average number of drugs per antibody may be calculated from the mixture by dual ELISA antibody assay, specific for antibody and specific for the drug. Individual ADC molecules may be identified in the mixture by mass spectroscopy.

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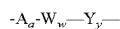
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copy, and separated by HPLC, e.g., hydrophobic interaction chromatography ("Effect of drug loading on the pharmacology, pharmacokinetics, and toxicity of an anti-CD30 antibody-drug conjugate", Hamblett, K. J., et al, Abstract No. 624, American Association for Cancer Research; 2004 Annual Meeting, Mar. 27-31, 2004, Proceedings of the AACR, Volume 45, March 2004; "Controlling the Location of Drug Attachment in Antibody-Drug Conjugates", Alley, S. C., et al, Abstract No. 627, American Association for Cancer Research; 2004 Annual Meeting, Mar. 27-31, 2004, Proceedings of the AACR, Volume 45, March 2004). Thus, a homogeneous ADC with a single loading value may be isolated from the conjugation mixture by electrophoresis or chromatography.

9.3 The Linker Unit

A "Linker unit" (LU) is a bifunctional compound which can be used to link a Drug unit and an Ligand unit to form Drug-Linker-Ligand Conjugates, or which are useful in the formation of immunoconjugates directed against tumor associated antigens. Such immunoconjugates allow the selective delivery of toxic drugs to tumor cells.

In one embodiment, the Linker unit of the Drug-Linker Compound and Drug-Linker-Ligand Conjugate has the formula:



wherein:

-A- is a Stretcher unit;

a is 0 or 1;

each —W— is independently an Amino Acid unit;

w is independently an integer ranging from 0 to 12;

—Y— is a Spacer unit; and

y is 0, 1 or 2.

In the Drug-Linker-Ligand Conjugate, the Linker is capable of linking the Drug moiety and the Ligand unit.

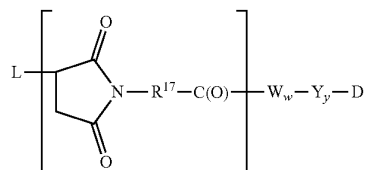
9.3.1 The Stretcher Unit

The Stretcher unit (-A-), when present, is capable of linking a Ligand unit to an amino acid unit (—W—). In this regard a Ligand (L) has a functional group that can form a bond with a functional group of a Stretcher. Useful functional groups that can be present on a ligand, either naturally or via chemical manipulation include, but are not limited to, sulfhydryl (—SH), amino, hydroxyl, carboxy, the anomeric hydroxyl group of a carbohydrate, and carboxyl. In one aspect, the Ligand functional groups are sulfhydryl and amino. Sulfhydryl groups can be generated by reduction of an intramolecular disulfide bond of a Ligand. Alternatively, sulfhydryl groups can be generated by reaction of an amino group of a lysine moiety of a Ligand using 2-iminothiolane (Traut's reagent) or another sulfhydryl generating reagent.

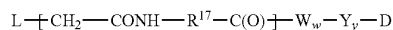
In one embodiment, the Stretcher unit forms a bond with a sulfur atom of the Ligand unit. The sulfur atom can be derived from a sulfhydryl group of a Ligand. Representative Stretcher units of this embodiment are depicted within the square brackets of Formulas Ma and IIb, wherein L, —W—, —Y—, —D, w and y are as defined above, and R¹⁷ is selected from —C₁-C₁₀ alkylene-, —C₃-C₈ carbocyclo-, —O-(C₁-C₈ alkyl)-, -arylene-, —C₁-C₁₀ alkylene-arylene-, -arylene-C₁-C₁₀ alkylene-, —C₁-C₁₀ alkylene-(C₃-C₈ carbocyclo)-, —(C₃-C₈ carbocyclo)-C₁-C₁₀ alkylene-, —C₃-C₈ heterocyclo-, —C₁-C₁₀ alkylene-(C₃-C₈ heterocyclo)-, —(C₃-C₈ heterocyclo)-C₁-C₁₀ alkylene-, —(CH₂CH₂O)_r—, and —(CH₂CH₂O)_r—CH₂—; and r is an integer ranging from 1-10. It is to be understood from all the

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exemplary embodiments of Formula Ia, such as that even where not denoted expressly, from 1 to 20 drug moieties are linked to a Ligand (p=1-20).

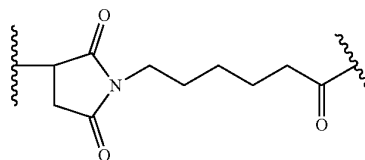


IIIa

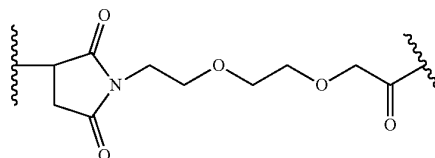


IIIb

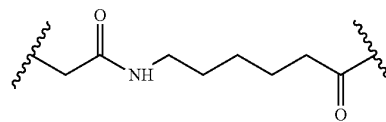
An illustrative Stretcher unit is that of Formula IIIa wherein R¹⁷ is —(CH₂)₅—:



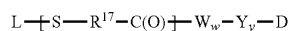
Another illustrative Stretcher unit is that of Formula IIIa wherein R¹⁷ is —(CH₂CH₂O)_r—CH₂—; and r is 2:



Still another illustrative Stretcher unit is that of Formula IIb wherein R¹⁷ is —(CH₂)₅—:



In another embodiment, the Stretcher unit is linked to the Ligand unit via a disulfide bond between a sulfur atom of the Ligand unit and a sulfur atom of the Stretcher unit. A representative Stretcher unit of this embodiment is depicted within the square brackets of Formula IV, wherein R¹⁷, L-, —W—, —Y—, —D, w and y are as defined above.



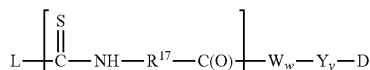
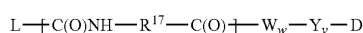
IV

In yet another embodiment, the reactive group of the Stretcher contains a reactive site that can form a bond with a primary or secondary amino group of a Ligand. Example of these reactive sites include, but are not limited to,

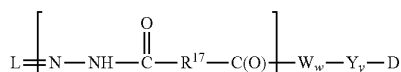
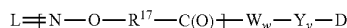
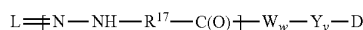
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activated esters such as succinimide esters, 4-nitrophenyl esters, pentafluorophenyl esters, tetrafluorophenyl esters, anhydrides, acid chlorides, sulfonyl chlorides, isocyanates and isothiocyanates. Representative Stretcher units of this embodiment are depicted within the square brackets of Formulas Va and Vb, wherein $-R^{17}-$, $-L-$, $-W-$, $-Y-$, $-D$, w and y are as defined above;

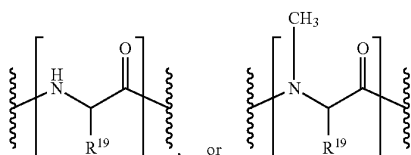


In yet another aspect, the reactive group of the Stretcher contains a reactive site that is reactive to a modified carbohydrate's ($-CHO$) group that can be present on a Ligand. For example, a carbohydrate can be mildly oxidized using a reagent such as sodium periodate and the resulting ($-CHO$) unit of the oxidized carbohydrate can be condensed with a Stretcher that contains a functionality such as a hydrazide, an oxime, a primary or secondary amine, a hydrazine, a thiosemicarbazone, a hydrazine carboxylate, and an arylhydrazide such as those described by Kaneko, T. et al. (1991) Bioconjugate Chem 2:133-41. Representative Stretcher units of this embodiment are depicted within the square brackets of Formulas Via, VIb, and VIc, wherein $-R^{17}-$, $-L-$, $-W-$, $-Y-$, $-D$, w and y are as defined above.



9.3.2 The Amino Acid Unit

The Amino Acid unit ($-W-$), when present, links the Stretcher unit to the Spacer unit if the Spacer unit is present, links the Stretcher unit to the Drug moiety if the Spacer unit is absent, and links the Ligand unit to the Drug unit if the Stretcher unit and Spacer unit are absent. W_w- is a dipeptide, tripeptide, tetrapeptide, pentapeptide, hexapeptide, heptapeptide, octapeptide, nonapeptide, decapeptide, undecapeptide or dodecapeptide unit. Each $-W-$ unit independently has the formula denoted below in the square brackets, and w is an integer ranging from 0 to 12:



wherein R^{19} is hydrogen, methyl, isopropyl, isobutyl, sec-butyl, benzyl, p-hydroxybenzyl, $-\text{CH}_2\text{OH}$, $-\text{CH}(\text{OH})\text{CH}_3$, $-\text{CH}_2\text{CH}_2\text{SCH}_3$, $-\text{CH}_2\text{CONH}_2$,

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$-\text{CH}_2\text{COOH}$, $-\text{CH}_2\text{CH}_2\text{CONH}_2$, $-\text{CH}_2\text{CH}_2\text{COOH}$, $-(\text{CH}_2)_3\text{NHC}(=\text{NH})\text{NH}_2$, $-(\text{CH}_2)_3\text{NH}_2$, $-(\text{CH}_2)_3\text{NHCOCH}_3$, $-(\text{CH}_2)_3\text{NHCHO}$, $-(\text{CH}_2)_4\text{NHC}(=\text{NH})\text{NH}_2$, $-(\text{CH}_2)_4\text{NH}_2$, $-(\text{CH}_2)_4\text{NHCOCH}_3$, $-(\text{CH}_2)_4\text{NHCHO}$, $-(\text{CH}_2)_3\text{NHCONH}_2$, $-(\text{CH}_2)_4\text{NHCONH}_2$, $-\text{CH}_2\text{CH}_2\text{CH}(\text{OH})\text{CH}_2\text{NH}_2$, 2-pyridylmethyl-, 3-pyridylmethyl-, 4-pyridylmethyl-, phenyl, cyclohexyl,

Va 10

Vb 15

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VIb

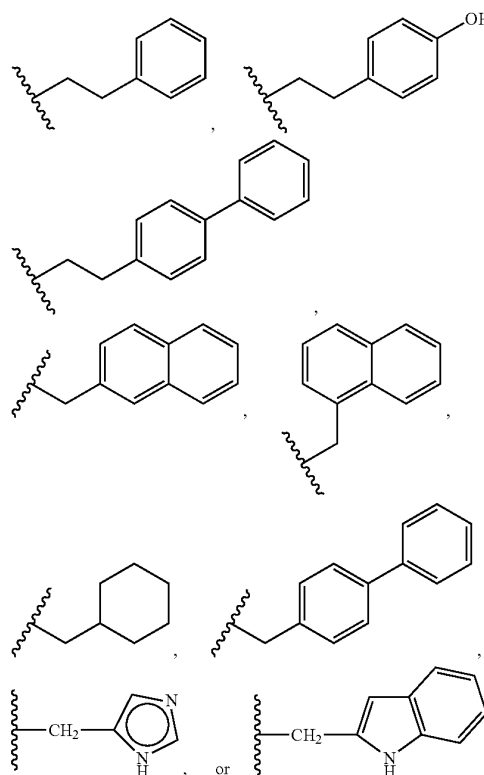
VIc 40

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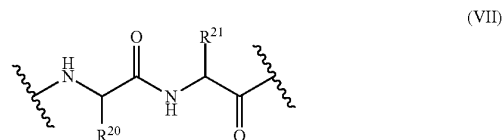
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The Amino Acid unit can be enzymatically cleaved by one or more enzymes, including a tumor-associated protease, to liberate the Drug unit ($-D$), which in one embodiment is protonated in vivo upon release to provide a Drug (D).

Illustrative W_w units are represented by formulas (VII)-(IX):



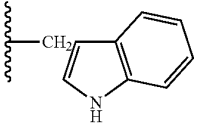
wherein R^{20} and R^{21} are as follows:

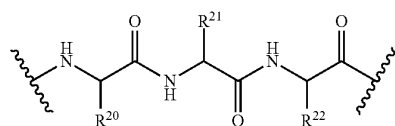
R^{20}	R^{21}
Benzyl	$(\text{CH}_2)_4\text{NH}_2$;
Methyl	$(\text{CH}_2)_4\text{NH}_2$;
isopropyl	$(\text{CH}_2)_4\text{NH}_2$;
isopropyl	$(\text{CH}_2)_3\text{NHCONH}_2$;
benzyl	$(\text{CH}_2)_3\text{NHCONH}_2$;

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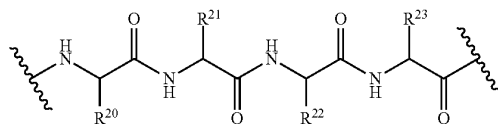
-continued

R ²⁰	R ²¹
isobutyl sec-butyl	(CH ₂) ₃ NHCONH ₂ ; (CH ₂) ₃ NHCONH ₂ ;
	(CH ₂) ₃ NHCONH ₂ ;
benzyl benzyl	methyl; and (CH ₂) ₃ NHC(=NH)NH ₂ ;



wherein R²⁰, R²¹ and R²² are as follows:

R ²⁰	R ²¹	R ²²
benzyl isopropyl H	Benzyl Benzyl Benzyl	(CH ₂) ₄ NH ₂ ; (CH ₂) ₄ NH ₂ ; and (CH ₂) ₄ NH ₂ ;



wherein R²⁰, R²¹, R²² and R²³ are as follows:

R ²⁰	R ²¹	R ²²	R ²³
H methyl	benzyl isobutyl	isobutyl methyl	H; and isobutyl.

Exemplary Amino Acid units include, but are not limited to, units of formula (VII) where: R²⁰ is benzyl and R²¹ is —(CH₂)₄NH₂; R²⁰ isopropyl and R²¹ is —(CH₂)₄NH₂; R²⁰ isobutyl and R²¹ is —(CH₂)₃NHCONH₂. Another exemplary Amino Acid unit is a unit of formula (VIII) wherein R²⁰ is benzyl, R²¹ is benzyl, and R²² is —(CH₂)₄NH₂.

Useful —W_w— units can be designed and optimized in their selectivity for enzymatic cleavage by a particular enzymes, for example, a tumor-associated protease. In one embodiment, a —W_w— unit is that whose cleavage is catalyzed by cathepsin B, C and D, or a plasmin protease.

In one embodiment, —W_w— is a dipeptide, tripeptide, tetrapeptide or pentapeptide.

When R¹⁹, R²⁰, R²¹, R²² or R²³ is other than hydrogen, the carbon atom to which R¹⁹, R²⁰, R²¹, R²² or R²³ is attached is chiral.

Each carbon atom to which R¹⁹, R²⁰, R²¹, R²² or R²³ is attached is independently in the (S) or (R) configuration.

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In one aspect of the Amino Acid unit, the Amino Acid unit is valine-citrulline. In another aspect, the Amino Acid unit is phenylalanine-lysine (i.e. fk). In yet another aspect of the Amino Acid unit, the Amino Acid unit is N-methylvaline-citrulline. In yet another aspect, the Amino Acid unit is 5-aminovaleric acid, homo phenylalanine lysine, tetraisoquinolinecarboxylate lysine, cyclohexylalanine lysine, isonepeccotic acid lysine, beta-alanine lysine, glycine serine valine glutamine and isonepeccotic acid.

In certain embodiments, the Amino Acid unit can comprise natural amino acids. In other embodiments, the Amino Acid unit can comprise non-natural amino acids.

9.3.3 The Spacer Unit

The Spacer unit (—Y—), when present, links an Amino Acid unit to the Drug moiety when an Amino Acid unit is present. Alternately, the Spacer unit links the Stretcher unit to the Drug moiety when the Amino Acid unit is absent. The Spacer unit also links the Drug moiety to the Ligand unit when both the Amino Acid unit and Stretcher unit are absent.

Spacer units are of two general types: self-immolative and non self-immolative. A non self-immolative Spacer unit is one in which part or all of the Spacer unit remains bound to the Drug moiety after cleavage, particularly enzymatic, of an Amino Acid unit from the Drug-Linker-Ligand Conjugate or the Drug-Linker Compound. Examples of a non self-immolative Spacer unit include, but are not limited to a (glycine-glycine) Spacer unit and a glycine Spacer unit (both depicted in FIG. 20) (infra). When an Exemplary Compound containing a glycine-glycine Spacer unit or a glycine Spacer unit undergoes enzymatic cleavage via a tumor-cell associated-protease, a cancer-cell-associated protease or a lymphocyte-associated protease, a glycine-glycine-Drug moiety or a glycine-Drug moiety is cleaved from L-A_a-Ww-. In one embodiment, an independent hydrolysis reaction takes place within the target cell, cleaving the glycine-Drug moiety bond and liberating the Drug.

In another embodiment, —Y_v— is a p-aminobenzyl alcohol (PAB) unit (see FIGS. 21 and 22) whose phenylene portion is substituted with Q_m wherein Q is —C₁-C₈ alkyl, —O—(C₁-C₈ alkyl), -halogen, -nitro or -cyano; and m is an integer ranging from 0-4.

In one embodiment, a non self-immolative Spacer unit (—Y—) is -Gly-Gly-.

In another embodiment, a non self-immolative the Spacer unit (—Y—) is -Gly-.

In one embodiment, a Drug-Linker Compound or a Drug-Linker Ligand Conjugate is provided in which the Spacer unit is absent (y=0), or a pharmaceutically acceptable salt or solvate thereof.

Alternatively, an Exemplary Compound containing a self-immolative Spacer unit can release -D without the need for a separate hydrolysis step. In this embodiment, —Y— is a PAB group that is linked to —W_w— via the amino nitrogen atom of the PAB group, and connected directly to -D via a carbonate, carbamate or ether group. Without being bound by any particular theory or mechanism, FIG. 21 depicts a possible mechanism of Drug release of a PAB group which is attached directly to -D via a carbamate or carbonate group espoused by Toki et al. (2002) J Org. Chem. 67:1866-1872.

In FIG. 21 Q is —C₁-C₈ alkyl, —O—(C₁-C₈ alkyl), -halogen, -nitro or -cyano; m is an integer ranging from 0-4; and p ranges from 1 to about 20.

Without being bound by any particular theory or mechanism, FIG. 22 depicts a possible mechanism of Drug release of a PAB group which is attached directly to -D via an ether or amine linkage.

In FIG. 22 Q is —C₁-C₈ alkyl, —O—(C₁-C₈ alkyl), -halogen, -nitro or -cyano; m is an integer ranging from 0-4; and p ranges from 1 to about 20.

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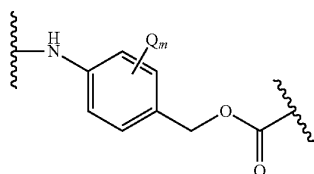
Other examples of self-immolative spacers include, but are not limited to, aromatic compounds that are electronically similar to the PAB group such as 2-aminoimidazol-5-methanol derivatives (Hay et al. (1999) *Bioorg. Med. Chem. Lett.* 9:2237) and ortho or para-aminobenzylacetals. Spacers can be used that undergo cyclization upon amide bond hydrolysis, such as substituted and unsubstituted 4-aminobutyric acid amides (Rodrigues et al., *Chemistry Biology*, 1995, 2, 223), appropriately substituted bicyclo[2.2.1] and bicyclo[2.2.2] ring systems (Storm, et al., *J. Amer. Chem. Soc.*, 1972, 94, 5815) and 2-aminophenylpropionic acid amides (Amsberry, et al., *J. Org. Chem.*, 1990, 55, 5867). Elimination of amine-containing drugs that are substituted at the α -position of glycine (Kingsbury, et al., *J. Med. Chem.*, 1984, 27, 1447) are also examples of self-immolative spacer useful in Exemplary Compounds.

In one embodiment, the Spacer unit is a branched bis(hydroxymethyl)styrene (BHMS) unit as depicted in FIG. 23, which can be used to incorporate and release multiple drugs.

In FIG. 23 Q is $-\text{C}_1\text{-C}_8$ alkyl, $-\text{O}-(\text{C}_1\text{-C}_8 \text{ alkyl})$, -halogen, -nitro or -cyano; m is an integer ranging from 0-4; n is 0 or 1; and p ranges raging from 1 to about 20.

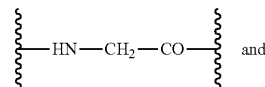
In one embodiment, the -D moieties are the same. In yet another embodiment, the -D moieties are different.

In one aspect, Spacer units ($-\text{Y}_y-$) are represented by Formulas (X)-(XII):

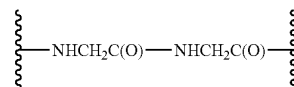


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wherein Q is —C₁-C₈ alkyl, —O—(C₁-C₈ alkyl), -halogen, -nitro or -cyano; and m is an integer ranging from 0-4;

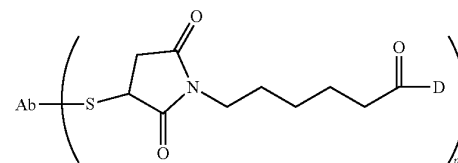
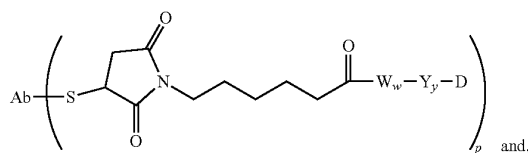


XI

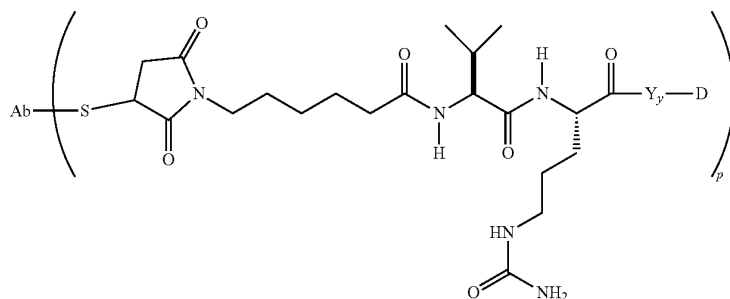
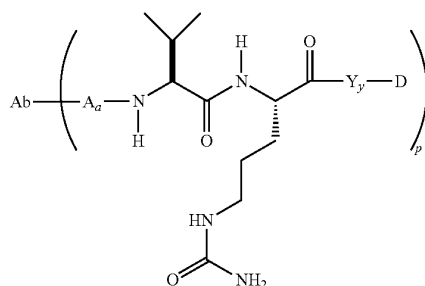


XII

Embodiments of the Formula Ia' and Ic antibody-drug conjugate compounds include:



wherein w and y are each 0,



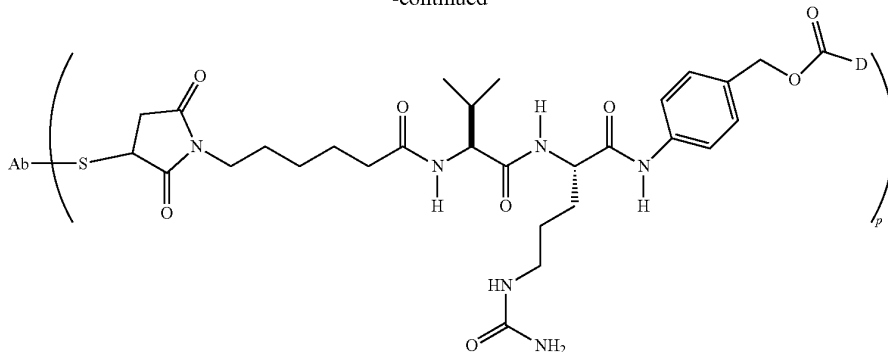
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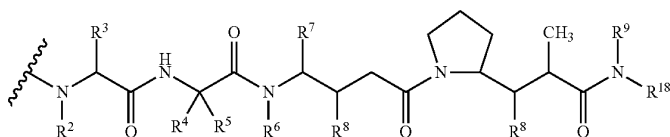
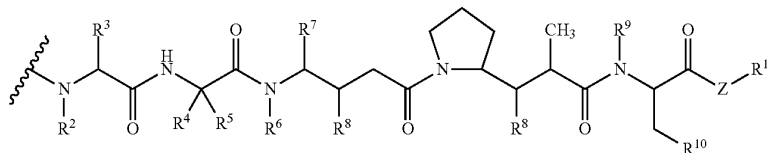


9.4 The Drug Unit (Moiety)

The drug moiety (D) of the antibody drug conjugates (ADC) are of the dolastatin/auristatin type (U.S. Pat. Nos. 5,635,483; 5,780,588) which have been shown to interfere with microtubule dynamics, GTP hydrolysis, and nuclear and cellular division (Woyke et al. (2001) Antimicrob. Agents and Chemother. 45(12):3580-3584) and have anti-cancer (U.S. Pat. No. 5,663,149) and antifungal activity (Pettit et al. (1998) Antimicrob. Agents Chemother. 42:2961-2965)

D is a Drug unit (moiety) having a nitrogen atom that can form a bond with the Spacer unit when $y=1$ or 2 , with the C-terminal carboxyl group of an Amino Acid unit when $y=0$, with the carboxyl group of a Stretcher unit when w and $y=0$, and with the carboxyl group of a Drug unit when a , w , and $y=0$. It is to be understood that the terms “drug unit” and “drug moiety” are synonymous and used interchangeably herein.

In one embodiment, -D is either formula D_E or D_F :

 D_E  D_F

wherein, independently at each location:

R^2 is selected from H and C_1 - C_8 alkyl;

R^3 is selected from H, C_1 - C_8 alkyl, C_3 - C_8 carbocycle, aryl, C_1 - C_8 alkyl-aryl, C_1 - C_8 alkyl- $(C_3$ - C_8 carbocycle), C_3 - C_8 heterocycle and C_1 - C_8 alkyl- $(C_3$ - C_8 heterocycle);

R^4 is selected from H, C_1 - C_8 alkyl, C_3 - C_8 carbocycle, aryl, C_1 - C_8 alkyl-aryl, C_1 - C_8 alkyl- $(C_3$ - C_8 carbocycle), C_3 - C_8 heterocycle and C_1 - C_8 alkyl- $(C_3$ - C_8 heterocycle);

each occurrence of R^{15} is independently H, COOH, $-(CH_2)_n-N(R^{16})_2$, $-(CH_2)_n-SO_3H$, or $-(CH_2)_n-SO_3-C_1$ - C_8 alkyl;

each occurrence of R^{16} is independently H, C_1 - C_8 alkyl, or $-(CH_2)_n-COOH$;

R^{18} is selected from $-C(R^8)_2-C(R^8)_2$ -aryl, $-C(R^8)_2-C(R^8)_2-(C_3$ - C_8 heterocycle), and $-C(R^8)_2-C(R^8)_2-(C_3$ - C_8 carbocycle); and

n is an integer ranging from 0 to 6.

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In one embodiment, R^3 , R^4 and R^7 are independently isopropyl or sec-butyl and R^5 is —H. In an exemplary embodiment, R^3 and R^4 are each isopropyl, R^5 is H, and R^7 is sec-butyl.

In another embodiment, R^2 and R^6 are each methyl, and R^9 is H.

In still another embodiment, each occurrence of R^8 is —OCH₃.

In an exemplary embodiment, R^3 and R^4 are each isopropyl, R^2 and R^6 are each methyl, R^5 is H, R^7 is sec-butyl, each occurrence of R^8 is —OCH₃, and R^9 is H.

In one embodiment, Z is —O— or —NH—.

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In one embodiment, R^{10} is aryl In an exemplary embodiment, R^{10} is -phenyl.

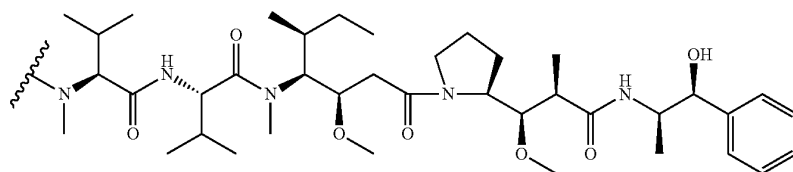
In an exemplary embodiment, when Z is —O—, R^{11} is H, methyl or t-butyl.

In one embodiment, when Z is —NH, R^{11} is —CH(R^{15})₂, wherein R^{15} is —(CH₂)_n—N(R^{16})₂, and R^{16} is —C₁–C₈ alkyl or —(CH₂)_n—COOH.

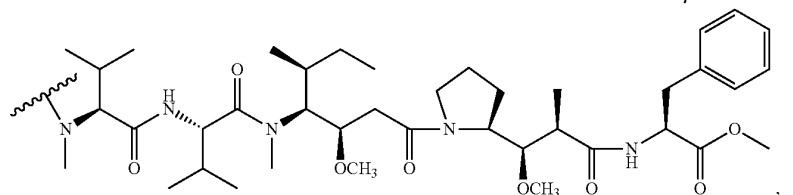
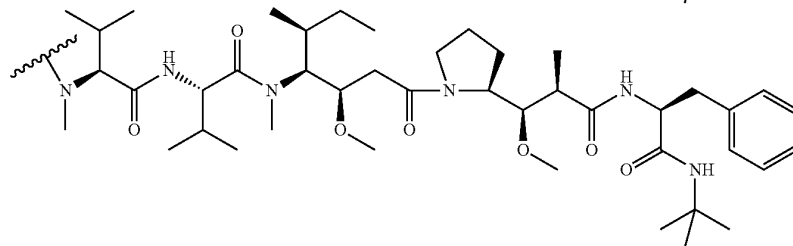
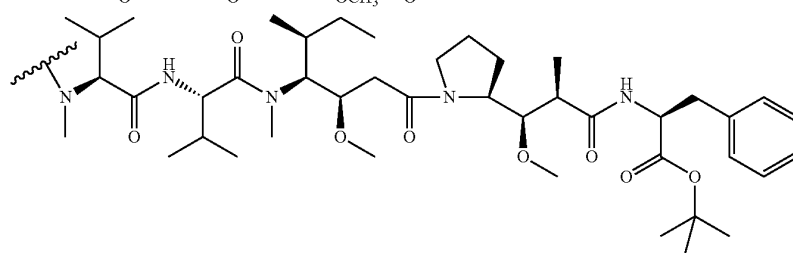
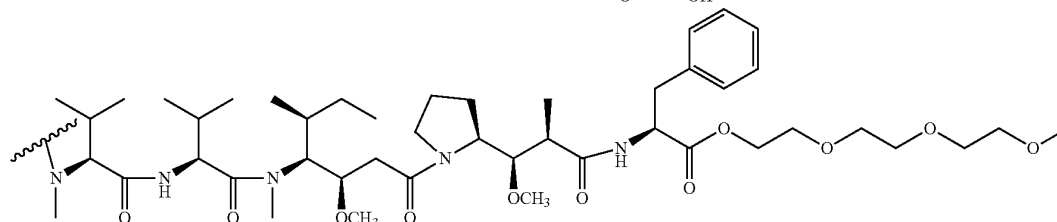
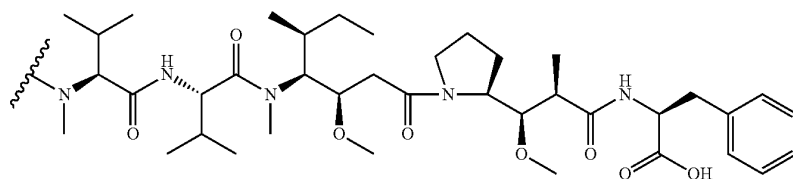
In another embodiment, when Z is —NH, R^{11} is —CH(R^{15})₂, wherein R^{15} is —(CH₂)_n—SO₃H.

Illustrative Drug units (-D) include the drug units having the following structures:

MMAE



MMAF

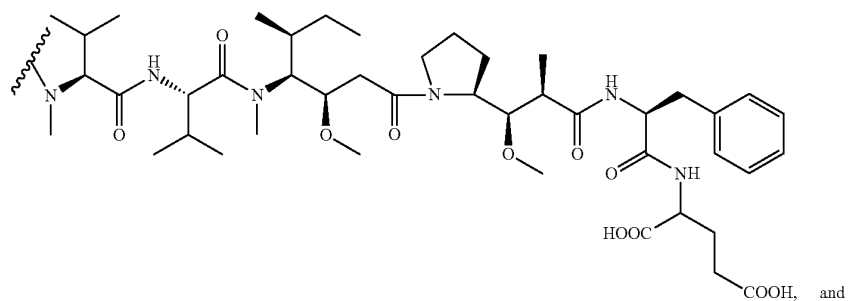
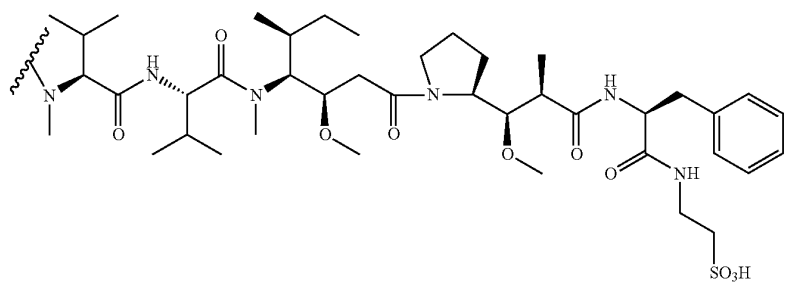
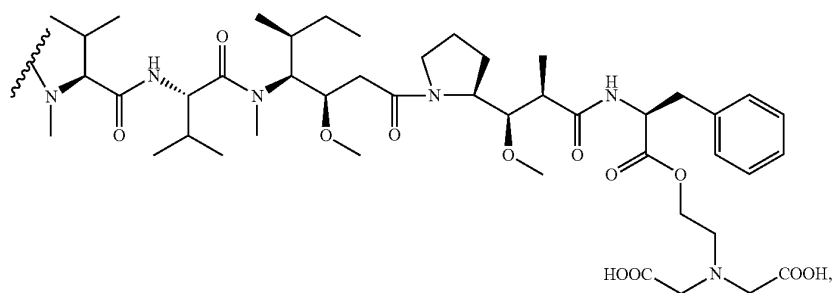
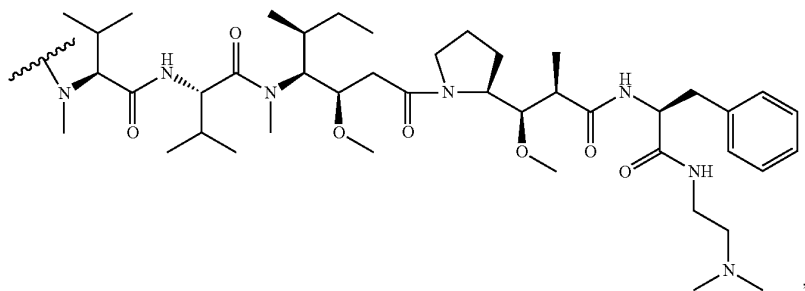


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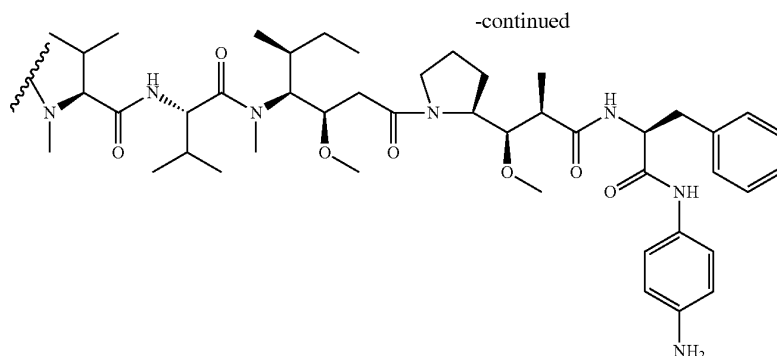
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and pharmaceutically acceptable salts or solvates thereof.

In one aspect, hydrophilic groups, such as but not limited to triethylene glycol esters (TEG), as shown above, can be attached to the Drug Unit at R¹¹. Without being bound by theory, the hydrophilic groups assist in the internalization and non-agglomeration of the Drug Unit.

9.5 The Ligand Unit

The Ligand unit (L-) includes within its scope any unit of a Ligand (L) that binds or reactively associates or complexes with a receptor, antigen or other receptive moiety associated with a given target-cell population. A Ligand is a molecule that binds to, complexes with, or reacts with a moiety of a cell population sought to be therapeutically or otherwise biologically modified. In one aspect, the Ligand unit acts to deliver the Drug unit to the particular target cell population with which the Ligand unit reacts. Such Ligands include, but are not limited to, large molecular weight proteins such as, for example, full-length antibodies, antibody fragments, smaller molecular weight proteins, polypeptide or peptides, lectins, glycoproteins, non-peptides, vitamins, nutrient-transport molecules (such as, but not limited to, transferrin), or any other cell binding molecule or substance.

A Ligand unit can form a bond to a Stretcher unit, an Amino Acid unit, a Spacer Unit, or a Drug Unit. A Ligand unit can form a bond to a Linker unit via a heteroatom of the Ligand. Heteroatoms that may be present on a Ligand unit include sulfur (in one embodiment, from a sulfhydryl group of a Ligand), oxygen (in one embodiment, from a carbonyl, carboxyl or hydroxyl group of a Ligand) and nitrogen (in one embodiment, from a primary or secondary amino group of a Ligand). These heteroatoms can be present on the Ligand in the Ligand's natural state, for example a naturally-occurring antibody, or can be introduced into the Ligand via chemical modification.

In one embodiment, a Ligand has a sulfhydryl group and the Ligand bonds to the Linker unit via the sulfhydryl group's sulfur atom.

In yet another aspect, the Ligand has one or more lysine residues that can be chemically modified to introduce one or more sulfhydryl groups. The Ligand unit bonds to the Linker unit via the sulfhydryl group's sulfur atom. The reagents that can be used to modify lysines include, but are not limited to, N-succinimidyl S-acetylthioacetate (SATA) and 2-Iminoethanol hydrochloride (Traut's Reagent).

In another embodiment, the Ligand can have one or more carbohydrate groups that can be chemically modified to have one or more sulfhydryl groups. The Ligand unit bonds to the Linker Unit, such as the Stretcher Unit, via the sulfhydryl group's sulfur atom.

In yet another embodiment, the Ligand can have one or more carbohydrate groups that can be oxidized to provide an aldehyde (—CHO) group (see, for e.g., Laguzza, et al., *J. Med. Chem.* 1989, 32(3), 548-55). The corresponding aldehyde can form a bond with a Reactive Site on a Stretcher. Reactive sites on a Stretcher that can react with a carbonyl group on a Ligand include, but are not limited to, hydrazine and hydroxylamine. Other protocols for the modification of proteins for the attachment or association of Drug Units are described in Coligan et al., *Current Protocols in Protein Science*, vol. 2, John Wiley & Sons (2002), incorporated herein by reference.

Useful non-immunoreactive protein, polypeptide, or peptide Ligands include, but are not limited to, transferrin, epidermal growth factors ("EGF"), bombesin, gastrin, gastrin-releasing peptide, platelet-derived growth factor, IL-2, IL-6, transforming growth factors ("TGF"), such as TGF- α and TGF- β , vaccinia growth factor ("VGF"), insulin and insulin-like growth factors I and II, lectins and apoprotein from low density lipoprotein.

Useful polyclonal antibodies are heterogeneous populations of antibody molecules derived from the sera of immunized animals. Various procedures well known in the art may be used for the production of polyclonal antibodies to an antigen-of-interest. For example, for the production of polyclonal antibodies, various host animals can be immunized by injection with an antigen of interest or derivative thereof, including but not limited to rabbits, mice, rats, and guinea pigs. Various adjuvants may be used to increase the immunological response, depending on the host species, and including but not limited to Freund's (complete and incomplete) adjuvant, mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and *Corynebacterium parvum*. Such adjuvants are also well known in the art.

Useful monoclonal antibodies are homogeneous populations of antibodies to a particular antigenic determinant (e.g., a cancer cell antigen, a viral antigen, a microbial antigen, a protein, a peptide, a carbohydrate, a chemical, nucleic acid, or fragments thereof). A monoclonal antibody (mAb) to an antigen-of-interest can be prepared by using any technique known in the art which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique originally described by Köhler and Milstein (1975, *Nature* 256, 495-497), the human B cell hybridoma technique (Kozbor et al., 1983, *Immunology Today* 4: 72),

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and the EBV-hybridoma technique (Cole et al., 1985, *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96). Such antibodies may be of any immunoglobulin class including IgG, IgM, IgE, IgA, and IgD and any subclass thereof. The hybridoma producing the mAbs of use in this invention may be cultivated in vitro or in vivo.

Useful monoclonal antibodies include, but are not limited to, human monoclonal antibodies, humanized monoclonal antibodies, antibody fragments, or chimeric human-mouse (or other species) monoclonal antibodies. Human monoclonal antibodies may be made by any of numerous techniques known in the art (e.g., Teng et al., 1983, *Proc. Natl. Acad. Sci. USA* 80, 7308-7312; Kozbor et al., 1983, *Immunology Today* 4, 72-79; and Olsson et al., 1982, *Meth. Enzymol.* 92, 3-16).

The antibody can also be a bispecific antibody. Methods for making bispecific antibodies are known in the art. Traditional production of full-length bispecific antibodies is based on the coexpression of two immunoglobulin heavy chain-light chain pairs, where the two chains have different specificities (Milstein et al., 1983, *Nature* 305:537-539). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. Similar procedures are disclosed in International Publication No. WO 93/08829, and in Traunecker et al., *EMBO J.* 10:3655-3659 (1991).

According to a different approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy chain constant domain, comprising at least part of the hinge, CH₂, and CH₃ regions. It is preferred to have the first heavy-chain constant region (CH₁) containing the site necessary for light chain binding, present in at least one of the fusions. Nucleic acids with sequences encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance.

In an embodiment of this approach, the bispecific antibodies have a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. This asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation (International Publication No. WO 94/04690) which is incorporated herein by reference in its entirety.

For further details for generating bispecific antibodies see, for example, Suresh et al., *Methods in Enzymology*, 1986, 121:210; Rodrigues et al., 1993, *J. of Immunology* 151: 6954-6961; Carter et al., 1992, *Bio/Technology* 10:163-167; Carter et al., 1995, *J. of Hematotherapy* 4:463-470; Merchant et al., 1998, *Nature Biotechnology* 16:677-681. Using

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such techniques, bispecific antibodies can be prepared for use in the treatment or prevention of disease as defined herein.

Bifunctional antibodies are also described, in European Patent Publication No. EPA 0 105 360. As disclosed in this reference, hybrid or bifunctional antibodies can be derived either biologically, i.e., by cell fusion techniques, or chemically, especially with cross-linking agents or disulfide-bridge forming reagents, and may comprise whole antibodies or fragments thereof. Methods for obtaining such hybrid antibodies are disclosed for example, in International Publication WO 83/03679, and European Patent Publication No. EPA 0 217 577, both of which are incorporated herein by reference.

The antibody can be a functionally active fragment, derivative or analog of an antibody that immunospecifically binds to cancer cell antigens, viral antigens, or microbial antigens or other antibodies bound to tumor cells or matrix. In this regard, "functionally active" means that the fragment, derivative or analog is able to elicit anti-anti-idiotypic antibodies that recognize the same antigen that the antibody from which the fragment, derivative or analog is derived recognized. Specifically, in an exemplary embodiment the antigenicity of the idiotype of the immunoglobulin molecule can be enhanced by deletion of framework and CDR sequences that are C-terminal to the CDR sequence that specifically recognizes the antigen. To determine which CDR sequences bind the antigen, synthetic peptides containing the CDR sequences can be used in binding assays with the antigen by any binding assay method known in the art (e.g., the BIA core assay) (See, for e.g., Kabat et al., 1991, *Sequences of Proteins of Immunological Interest*, Fifth Edition, National Institute of Health, Bethesda, Md.; Kabat E et al., 1980, *J. of Immunology* 125(3):961-969).

Other useful antibodies include fragments of antibodies such as, but not limited to, F(ab')₂ fragments, which contain the variable region, the light chain constant region and the CH₁ domain of the heavy chain can be produced by pepsin digestion of the antibody molecule, and Fab fragments, which can be generated by reducing the disulfide bridges of the F(ab')₂ fragments. Other useful antibodies are heavy chain and light chain dimers of antibodies, or any minimal fragment thereof such as Fvs or single chain antibodies (SCAs) (e.g., as described in U.S. Pat. No. 4,946,778; Bird, 1988, *Science* 242:423-42; Huston et al., 1988, *Proc. Natl. Acad. Sci. USA* 85:5879-5883; and Ward et al., 1989, *Nature* 334:544-54), or any other molecule with the same specificity as the antibody.

Additionally, recombinant antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, which can be made using standard recombinant DNA techniques, are useful antibodies. A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine monoclonal and human immunoglobulin constant regions. (See, e.g., Cabilly et al., U.S. Pat. No. 4,816,567; and Boss et al., U.S. Pat. No. 4,816,397, which are incorporated herein by reference in their entirety.) Humanized antibodies are antibody molecules from non-human species having one or more complementarity determining regions (CDRs) from the non-human species and a framework region from a human immunoglobulin molecule. (See, e.g., Queen, U.S. Pat. No. 5,585,089, which is incorporated herein by reference in its entirety.) Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art, for example using methods

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described in International Publication No. WO 87/02671; European Patent Publication No. 184,187; European Patent Publication No. 171496; European Patent Publication No. 173494; International Publication No. WO 86/01533; U.S. Pat. No. 4,816,567; European Patent Publication No. 12,023; Berter et al., 1988, Science 240:1041-1043; Liu et al., 1987, Proc. Natl. Acad. Sci. USA 84:3439-3443; Liu et al., 1987, J. Immunol. 139:3521-3526; Sun et al., 1987, Proc. Natl. Acad. Sci. USA 84:214-218; Nishimura et al., 1987, Cancer. Res. 47:999-1005; Wood et al., 1985, Nature 314:446-449; and Shaw et al., 1988, J. Natl. Cancer Inst. 80:1553-1559; Morrison, 1985, Science 229:1202-1207; Oi et al., 1986, BioTechniques 4:214; U.S. Pat. No. 5,225,539; Jones et al., 1986, Nature 321:552-525; Verhoeven et al. (1988) Science 239:1534; and Beidler et al., 1988, J. Immunol. 141:4053-4060; each of which is incorporated herein by reference in its entirety.

Completely human antibodies are particularly desirable and can be produced using transgenic mice that are incapable of expressing endogenous immunoglobulin heavy and light chains genes, but which can express human heavy and light chain genes. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a polypeptide of the invention. Monoclonal antibodies directed against the antigen can be obtained using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar (1995, *Int. Rev. Immunol.* 13:65-93). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies. See, e.g., U.S. Pat. Nos. 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; each of which is incorporated herein by reference in its entirety. Other human antibodies can be obtained commercially from, for example, Abgenix, Inc. (Freemont, Calif.) and Genpharm (San Jose, Calif.).

Completely human antibodies that recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers et al. (1994) *Biotechnology* 12:899-903). Human antibodies can also be produced using various techniques known in the art, including phage display libraries (Hoogenboom and Winter, J. Mol. Biol., 227:381 (1991); Marks et al., J. Mol. Biol., 222:581 (1991); Quan, M. P. and Carter, P. 2002. *The rise of monoclonal antibodies as therapeutics*. In *Anti-IgE and Allergic Disease*, Jardieu, P. M. and Fick Jr., R. B., eds., Marcel Dekker, New York, N.Y., Chapter 20, pp. 427-469).

In other embodiments, the antibody is a fusion protein of an antibody, or a functionally active fragment thereof, for example in which the antibody is fused via a covalent bond (e.g., a peptide bond), at either the N-terminus or the C-terminus to an amino acid sequence of another protein (or portion thereof, preferably at least 10, 20 or 50 amino acid portion of the protein) that is not the antibody. Preferably, the antibody or fragment thereof is covalently linked to the other protein at the N-terminus of the constant domain.

Antibodies include analogs and derivatives that are either modified, i.e., by the covalent attachment of any type of molecule as long as such covalent attachment permits the

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antibody to retain its antigen binding immunospecificity. For example, but not by way of limitation, the derivatives and analogs of the antibodies include those that have been further modified, e.g., by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular antibody unit or other protein, etc. Any of numerous chemical modifications can be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis in the presence of tunicamycin, etc. Additionally, the analog or derivative can contain one or more unnatural amino acids.

The antibodies include antibodies having modifications (e.g., substitutions, deletions or additions) in amino acid residues that interact with Fc receptors. In particular, antibodies include antibodies having modifications in amino acid residues identified as involved in the interaction between the anti-Fc domain and the FcRn receptor (see, e.g., International Publication No. WO 97/34631, which is incorporated herein by reference in its entirety). Antibodies immunospecific for a cancer cell antigen can be obtained commercially, for example, from Genentech (San Francisco, Calif.) or produced by any method known to one of skill in the art such as, e.g., chemical synthesis or recombinant expression techniques. The nucleotide sequence encoding antibodies immunospecific for a cancer cell antigen can be obtained, e.g., from the GenBank database or a database like it, the literature publications, or by routine cloning and sequencing.

In a specific embodiment, known antibodies for the treatment or prevention of cancer can be used. Antibodies immunospecific for a cancer cell antigen can be obtained commercially or produced by any method known to one of skill in the art such as, e.g., recombinant expression techniques. The nucleotide sequence encoding antibodies immunospecific for a cancer cell antigen can be obtained, e.g., from the GenBank database or a database like it, the literature publications, or by routine cloning and sequencing. Examples of antibodies available for the treatment of cancer include, but are not limited to, humanized anti-HER2 monoclonal antibody, HERCEPTIN® (trastuzumab; Genentech) for the treatment of patients with metastatic breast cancer; RITUXAN® (rituximab; Genentech) which is a chimeric anti-CD20 monoclonal antibody for the treatment of patients with non-Hodgkin's lymphoma; OvaRex (AltaRex Corporation, MA) which is a murine antibody for the treatment of ovarian cancer; Panorex (Glaxo Wellcome, NC) which is a murine IgG_{2a} antibody for the treatment of colorectal cancer; Cetuximab Erbitux (Imclone Systems Inc., NY) which is an anti-EGFR IgG chimeric antibody for the treatment of epidermal growth factor positive cancers, such as head and neck cancer; Vitaxin (MedImmune, Inc., MD) which is a humanized antibody for the treatment of sarcoma; Campath UH (Leukosite, MA) which is a humanized IgG₁ antibody for the treatment of chronic lymphocytic leukemia (CLL); Smart MI95 (Protein Design Labs, Inc., CA) which is a humanized anti-CD33 IgG antibody for the treatment of acute myeloid leukemia (AML); LymphoCide (Immuno-medics, Inc., NJ) which is a humanized anti-CD22 IgG antibody for the treatment of non-Hodgkin's lymphoma; Smart ID10 (Protein Design Labs, Inc., CA) which is a humanized anti-HLA-DR antibody for the treatment of non-Hodgkin's lymphoma; Oncolym (Techniclone, Inc., CA) which is a radiolabeled murine anti-HLA-Dr10 antibody for the treatment of non-Hodgkin's lymphoma; Allo-mune (BioTransplant, CA) which is a humanized anti-CD2 mAb for the treatment of Hodgkin's Disease or non-Hodg-

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kin's lymphoma; Avastin (Genentech, Inc., CA) which is an anti-VEGF humanized antibody for the treatment of lung and colorectal cancers; Epratuzamab (Immunomedics, Inc., NJ and Amgen, CA) which is an anti-CD22 antibody for the treatment of non-Hodgkin's lymphoma; and CEAcide (Immunomedics, NJ) which is a humanized anti-CEA antibody for the treatment of colorectal cancer.

Other antibodies useful in the treatment of cancer include, but are not limited to, antibodies against the following antigens: CA125 (ovarian), CA15-3 (carcinomas), CA19-9 (carcinomas), L6 (carcinomas), Lewis Y (carcinomas), Lewis X (carcinomas), alpha fetoprotein (carcinomas), CA 242 (colorectal), placental alkaline phosphatase (carcinomas), prostate specific antigen (prostate), prostatic acid phosphatase (prostate), epidermal growth factor (carcinomas), MAGE-1 (carcinomas), MAGE-2 (carcinomas), MAGE-3 (carcinomas), MAGE-4 (carcinomas), anti-transferrin receptor (carcinomas), p97 (melanoma), MUC1-KLH (breast cancer), CEA (colorectal), gp100 (melanoma), MART1 (melanoma), PSA (prostate), IL-2 receptor (T-cell leukemia and lymphomas), CD20 (non-Hodgkin's lymphoma), CD52 (leukemia), CD33 (leukemia), CD22 (lymphoma), human chorionic gonadotropin (carcinoma), CD38 (multiple myeloma), CD40 (lymphoma), mucin (carcinomas), P21 (carcinomas), MPG (melanoma), and Neu oncogene product (carcinomas). Some specific, useful antibodies include, but are not limited to, BR96 mAb (Trail, P. A., Willner, D., Lasch, S. J., Henderson, A. J., Hofstead, S. J., Casazza, A. M., Firestone, R. A., Hellstrom, I., Hellstrom, K. E., "Cure of Xenografted Human Carcinomas by BR96-Doxorubicin Immunoconjugates" *Science* 1993, 261, 212-215), BR64 (Trail, P. A., Willner, D., Knipe, J., Henderson, A. J., Lasch, S. J., Zoeckler, M. E., Trailsmith, M. D., Doyle, T. W., King, H. D., Casazza, A. M., Braslawsky, G. R., Brown, J. P., Hofstead, S. J., (Greenfield, R. S., Firestone, R. A., Mosure, K., Kadow, D. F., Yang, M. B., Hellstrom, K. E., and Hellstrom, I. "Effect of Linker Variation on the Stability, Potency, and Efficacy of Carcinoma-reactive BR64-Doxorubicin Immunoconjugates" *Cancer Research* 1997, 57, 100-105, mAbs against the CD40 antigen, such as S2C₆ mAb (Francisco, J. A., Donaldson, K. L., Chace, D., Siegall, C. B., and Wahl, A. F. "Agonistic properties and in vivo antitumor activity of the anti-CD-40 antibody, SGN-14" *Cancer Res.* 2000, 60, 3225-3231), mAbs against the CD70 antigen, such as 1F6 mAb and 2F2 mAb, and mAbs against the CD30 antigen, such as AC10 (Bowen, M. A., Olsen, K. J., Cheng, L., Avila, D., and Podack, E. R. "Functional effects of CD30 on a large granular lymphoma cell line YT" *J. Immunol.*, 151, 5896-5906, 1993: Wahl et al., 2002 *Cancer Res.* 62(13):3736-42). Many other internalizing antibodies that bind to tumor associated antigens can be used and have been reviewed (Franke, A. E., Sievers, E. L., and Scheinberg, D. A., "Cell surface receptor-targeted therapy of acute myeloid leukemia: a review" *Cancer Biother Radiopharm.* 2000, 15, 459-76; Murray, J. L., "Monoclonal antibody treatment of solid tumors: a coming of age" *Semin Oncol.* 2000, 27, 64-70; Breitling, F., and Dubel, S., *Recombinant Antibodies*, John Wiley, and Sons, New York, 1998).

In certain embodiments, the antibody is not Trastuzumab (full length, humanized anti-HER2 (MW 145167)), HerceptinF(ab')₂ (derived from anti-HER2 enzymatically (MW 100000)), 4D5 (full-length, murine antiHER2, from hybridoma), rhu4D5 (transiently expressed, full-length humanized antibody), rhuFab4D5 (recombinant humanized Fab (MW 47738)), 4D5Fc8 (full-length, murine antiHER2, with mutated FcRn binding domain), or Hg ("Hingeless"

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full-length humanized 4D5, with heavy chain hinge cysteines mutated to serines. Expressed in *E. coli* (therefore non-glycosylated)).

In another specific embodiment, known antibodies for the treatment or prevention of an autoimmune disease are used in accordance with the compositions and methods of the invention. Antibodies immunospecific for an antigen of a cell that is responsible for producing autoimmune antibodies can be obtained from any organization (e.g., a university scientist or a company) or produced by any method known to one of skill in the art such as, e.g., chemical synthesis or recombinant expression techniques. In another embodiment, useful antibodies are immunospecific for the treatment of autoimmune diseases include, but are not limited to, Anti-Nuclear Antibody; Anti-ds DNA; Anti-ss DNA, Anti-Cardiolipin Antibody IgM, IgG; Anti-Phospholipid Antibody IgM, IgG; Anti-SM Antibody; Anti-Mitochondrial Antibody; Thyroid Antibody; Microsomal Antibody; Thyroglobulin Antibody; Anti-SCL-70; Anti-Jo; Anti-U₁RNP; Anti-La/SSB; Anti-SSA; Anti-SSB; Anti-Perital Cells Antibody; Anti-Histones; Anti-RNP; C-ANCA; P-ANCA; Anti-centromere; Anti-Fibrillarin, and Anti-GBM Antibody.

In certain embodiments, useful antibodies can bind to both a receptor or a receptor complex expressed on an activated lymphocyte. The receptor or receptor complex can comprise an immunoglobulin gene superfamily member, a TNF receptor superfamily member, an integrin, a cytokine receptor, a chemokine receptor, a major histocompatibility protein, a lectin, or a complement control protein. Non-limiting examples of suitable immunoglobulin superfamily members are CD2, CD3, CD4, CD8, CD19, CD22, CD28, CD79, CD90, CD152/CTLA-4, PD-1, and ICOS. Non-limiting examples of suitable TNF receptor superfamily members are CD27, CD40, CD95/Fas, CD134/OX40, CD137/4-1BB, TNF-R1, TNFR-2, RANK, TACI, BCMA, osteoprotegerin, Apo2/TRAIL-R1, TRAIL-R2, TRAIL-R3, TRAIL-R4, and APO-3. Non-limiting examples of suitable integrins are CD11a, CD11b, CD11c, CD18, CD29, CD41, CD49a, CD49b, CD49c, CD49d, CD49e, CD49f, CD103, and CD104. Non-limiting examples of suitable lectins are C-type, S-type, and I-type lectin.

In one embodiment, the Ligand binds to an activated lymphocyte that is associated with an autoimmune disease.

In another specific embodiment, useful Ligands immunospecific for a viral or a microbial antigen are monoclonal antibodies. The antibodies may be chimeric, humanized or human monoclonal antibodies. As used herein, the term "viral antigen" includes, but is not limited to, any viral peptide, polypeptide protein (e.g., HIV gp120, HIV nef, RSV F glycoprotein, influenza virus neuraminidase, influenza virus hemagglutinin, HTLV tax, herpes simplex virus glycoprotein (e.g., gB, gC, gD, and gE) and hepatitis B surface antigen) that is capable of eliciting an immune response. As used herein, the term "microbial antigen" includes, but is not limited to, any microbial peptide, polypeptide, protein, saccharide, polysaccharide, or lipid molecule (e.g., a bacterial, fungi, pathogenic protozoa, or yeast polypeptide including, e.g., LPS and capsular polysaccharide 5/8) that is capable of eliciting an immune response.

Antibodies immunospecific for a viral or microbial antigen can be obtained commercially, for example, from BD Biosciences (San Francisco, Calif.), Chemicon International, Inc. (Temecula, Calif.), or Vector Laboratories, Inc. (Burlingame, Calif.) or produced by any method known to one of skill in the art such as, e.g., chemical synthesis or recombinant expression techniques. The nucleotide sequence encoding antibodies that are immunospecific for a

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viral or microbial antigen can be obtained, e.g., from the GenBank database or a database like it, literature publications, or by routine cloning and sequencing.

In a specific embodiment, useful Ligands are those that are useful for the treatment or prevention of viral or microbial infection in accordance with the methods disclosed herein. Examples of antibodies available useful for the treatment of viral infection or microbial infection include, but are not limited to, SYNAGIS (MedImmune, Inc., MD) which is a humanized anti-respiratory syncytial virus (RSV) monoclonal antibody useful for the treatment of patients with RSV infection; PRO542 (Progenics) which is a CD4 fusion antibody useful for the treatment of HIV infection; OSTAVIR (Protein Design Labs, Inc., CA) which is a human antibody useful for the treatment of hepatitis B virus; PROTOVIR (Protein Design Labs, Inc., CA) which is a humanized IgG₁ antibody useful for the treatment of cytomegalovirus (CMV); and anti-LPS antibodies.

Other antibodies useful in the treatment of infectious diseases include, but are not limited to, antibodies against the antigens from pathogenic strains of bacteria (*Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Corynebacterium diphtheriae*, *Clostridium botulinum*, *Clostridium perfringens*, *Clostridium tetani*, *Hemophilus influenzae*, *Klebsiella pneumoniae*, *Klebsiella ozaenas*, *Klebsiella rhinoscleromatis*, *Staphylococcus aureus*, *Vibrio cholerae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Campylobacter (Vibrio) fetus*, *Aeromonas hydrophila*, *Bacillus cereus*, *Edwardsiella tarda*, *Yersinia enterocolitica*, *Yersinia pestis*, *Yersinia pseudotuberculosis*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, *Salmonella typhimurium*, *Treponema pallidum*, *Treponema pertenue*, *Treponema caratenum*, *Borrelia vincentii*, *Borrelia burgdorferi*, *Leptospira icterohemorrhagiae*, *Mycobacterium tuberculosis*, *Pneumocystis carinii*, *Francisella tularensis*, *Brucella abortus*, *Brucella suis*, *Brucella melitensis*, *Mycoplasma* spp., *Rickettsia prowazekii*, *Rickettsia tsutsugumushi*, *Chlamydia* spp.); pathogenic fungi (*Coccidioides immitis*, *Aspergillus fumigatus*, *Candida albicans*, *Blastomyces dermatitidis*, *Cryptococcus neoformans*, *Histoplasma capsulatum*); protozoa (*Entamoeba histolytica*, *Toxoplasma gondii*, *Trichomonas tenax*, *Trichomonas hominis*, *Trichomonas vaginalis*, *Trypanosoma gambiense*, *Trypanosoma rhodesiense*, *Trypanosoma cruzi*, *Leishmania donovani*, *Leishmania tropica*, *Leishmania braziliensis*, *Pneumocystis pneumonia*, *Plasmodium vivax*, *Plasmodium falciparum*, *Plasmodium malariae*); or Helminths (*Enterobius vermicularis*, *Trichuris trichiura*, *Ascaris lumbricoides*, *Trichinella spiralis*, *Strongyloides stercoralis*, *Schistosoma japonicum*, *Schistosoma mansoni*, *Schistosoma haematobium*, and hookworms).

Other antibodies useful in this invention for treatment of viral disease include, but are not limited to, antibodies against antigens of pathogenic viruses, including as examples and not by limitation: Poxviridae, Herpesviridae, Herpes Simplex virus 1, Herpes Simplex virus 2, Adenoviridae, Papovaviridae, Enteroviridae, Picornaviridae, Parvoviridae, Reoviridae, Retroviridae, influenza viruses, parainfluenza viruses, mumps, measles, respiratory syncytial virus, rubella, Arboviridae, Rhabdoviridae, Arenaviridae, Hepatitis A virus, Hepatitis B virus, Hepatitis C virus, Hepatitis E virus, Non-A/Non-B Hepatitis virus, Rhinoviridae, Coronaviridae, Rotoviridae, and Human Immunodeficiency Virus.

In attempts to discover effective cellular targets for cancer diagnosis and therapy, researchers have sought to identify transmembrane or otherwise tumor-associated polypeptides

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that are specifically expressed on the surface of one or more particular type(s) of cancer cell as compared to one or more normal non-cancerous cell(s). Often, such tumor-associated polypeptides are more abundantly expressed on the surface of the cancer cells as compared to on the surface of the non-cancerous cells. The identification of such tumor-associated cell surface antigen polypeptides has given rise to the ability to specifically target cancer cells for destruction via antibody-based therapies.

Antibodies which comprise Ab in Formula Ic antibody drug conjugates (ADC) and which may be useful in the treatment of cancer include, but are not limited to, antibodies against tumor-associated antigens (TAA). Such tumor-associated antigens are known in the art, and can be prepared for use in generating antibodies using methods and information which are well known in the art. Examples of TAA include (1)-(35), but are not limited to TAA (1)-(35) listed below. For convenience, information relating to these antigens, all of which are known in the art, is listed below and includes names, alternative names, Genbank accession numbers and primary reference(s). Tumor-associated antigens targeted by antibodies include all amino acid sequence variants and isoforms possessing at least about 70%, 80%, 85%, 90%, or 95% sequence identity relative to the sequences identified in the corresponding sequences listed (SEQ ID NOS: 1-35) or the sequences identified in the cited references. In some embodiments, TAA having amino acid sequence variants exhibit substantially the same biological properties or characteristics as a TAA having the sequence found in the corresponding sequences listed (SEQ ID NOS: 1-35). For example, a TAA having a variant sequence generally is able to bind specifically to an antibody that binds specifically to the TAA with the corresponding sequence listed. The sequences and disclosure specifically recited herein are expressly incorporated by reference.

Tumor-Associated Antigens (1)-(35):

(1) BMPR1B (bone morphogenetic protein receptor-type IB, Genbank accession no. NM_001203, ten Dijke, P., et al. Science 264 (5155):101-104 (1994), *Oncogene* 14 (11): 1377-1382 (1997)); WO2004063362 (claim 2); WO2003042661 (claim 12); US2003134790-A1 (Page 38-39); WO2002102235 (claim 13; Page 296); WO2003055443 (Page 91-92); WO200299122 (Example 2; Page 528-530); WO2003029421 (claim 6); WO2003024392 (claim 2; FIG. 112); WO200298358 (claim 1; Page 183); WO200254940 (Page 100-101); WO200259377 (Page 349-350); WO200230268 (claim 27; Page 376); WO200148204 (Example; FIG. 4)

NP_001194 bone morphogenetic protein receptor, type D3/pid=NP_001194.1—

-Cross-references: MIM:603248; NP_001194.1; NM_001203_1

502 aa

(SEQ ID NO: 1)

MLLRSGAKLVNIGIKKEDGESTAPTRPKVLRCCKHHCPEDSVNNICSTD
GYCFTMIEEDSGLPVVTSGCLGLESGDFQCRDTPIPHQRRSIECCTERN
ECNKDLHPTLPPLKNRDEVDGPPIHRRALLISVTVCSLLLVLIILFCYFRY
KRQETRPYSIGLEQDETYIPGESLRDLIEQSQSSGSGSLPLLQRTI
AKQIQMVKQIGKGRYGEVWMGKWRGEKVAVKVFETTEASWFRETEIYQT
VLMRHNILGFIADIKGTGSWTQLYLI TDYHENGSLYDLKSTTLDKAS
MLKLAYS SVSGLCHLHTEIFSTQGKPAIAHRDLKSKNVLKKNKGTCCIAD

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LGLAVKFISDTNEVDIPPNTRVGTRKRYMPPEVLDESILNRNHFQSYIMADM
 YSFGILILWEVARRCVSGGIVEEYQLPYHDLVPSDPSYEDMREIVCIKKLR
 PSFPNRWSSDECLRQMGKLMTECAHNAPASRLTALRVKKTAKMSESQDI
 KL

(2) E16 (LAT1, SLC7A5, Genbank accession no. NM_003486);

Biochem. Biophys. Res. Commun. 255 (2), 283-288 (1999), Nature 395 (6699):288-291 (1998), Gaugitsch, H. W., et al. (1992) J. Biol. Chem. 267 (16):11267-11273; WO2004048938 (Example 2); WO2004032842 (Example IV); WO2003042661 (claim 12); WO2003016475 (claim 1); WO200278524 (Example 2); WO200299074 (claim 19; Page 127-129); WO200286443 (claim 27; Pages 222, 393); WO2003003906 (claim 10; Page 293); WO200264798 (claim 33; Page 93-95); WO200014228 (claim 5; Page 133-136); US2003224454 (FIG. 3); WO2003025138 (claim 12; Page 150);

NP_003477 solute carrier family 7 (cationic amino acid transporter, y+ system), member 5/pid=NP_003477.3—*Homo sapiens*

Cross-references: MIM:600182; NP_003477.3; NM_015923; NM_003486_1

507 aa

(SEQ ID NO: 2)

MAGAGPKRRALAAPAAEEKEEAREKMLAASADGSAPAGEGEGVTLQRNI
 TLLNGVAIVIGTTIIGSGIFVPTPTGVLEAGSPGLALVVAACGVFSIVGA
 LCYAEGLTTISKSGGDYAYMLEVYGLPAFLKLWIELLIIRPSSQYIVAL
 VFATYLLKPLFPTCPVEEAAKLVAACLVLLLTAVNCYSVKAATRVQDAF
 AAKLLALALAIILLGFVQIGKGVVSNLDPNFSFEGTKLDVGNIVLALYSG
 LFAYGGWNYLNFVTEEMINPYRNLPLAIIISLPITLVYVLTNLAYFTTL
 STEQMLSSAEAVDFGNVHLGVMSWIIIPVFGVLSVSGVNGSLFTSSRLF
 FVGSREGHLPSILSMIHPQLLTPVPSLVFTCVMTLLYAFSKDIFSVINFF

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-continued

SFFNWLCLVALAIIGMIWLRHRKPELERPIKVNLAALPVFFILACLFLIAVS
 FWKTPVECGIGFTIILSGLPVYFVGWKNKPKWLLQGIFSTTVLCQKLM
 QVVPQET

(3) STEAP1 (six transmembrane epithelial antigen of prostate, Genbank accession no. NM_012449

Cancer Res. 61 (15), 5857-5860 (2001), Hubert, R. S., et al. (1999) Proc. Natl. Acad. Sci. USA. 96 (25):14523-14528; WO2004065577 (claim 6); WO2004027049 (FIG. 1L); EP1394274 (Example 11); WO2004016225 (claim 2); WO2003042661 (claim 12); US2003157089 (Example 5); US2003185830 (Example 5); US2003064397 (FIG. 2); WO200289747 (Example 5; Page 618-619); WO2003022995 (Example 9; FIG. 13A, Example 53; Page 173, Example 2; FIG. 2A); NP_036581 six transmembrane epithelial antigen of the prostate

Cross-references: MIM:604415; NP_036581.1; NM_012449_1

339 aa

(SEQ ID NO: 3)

MESRKDITNQEELWKMKPRRNLLEDDYLHKDTGETSMLKRPVLLHLHQTA
 HADEFDCPSELQHTQELFPQWHLPIKIAAIIASLTFLYTLREVIHPLAT
 SHQQYFYKIPILVINKVLPVVSITLLALVYLPGVIAAIVQLHNGTKYKKF
 PHWLDKWMLTRKQFGLLSFFFAVLHAIYSLSYPMRRSRYKLLNWAYQQV
 QONKEDAWIEHDVWRMEIYVSLGIVGLAILALLAVTSIPSVSDSLWREF
 HYIQSKLGIVSLLLGTIHALIFAWNKWIDIKQFVWYTPPTFMIAVFLPIV
 VLIFKSLFLPCLRKILKIRHGWEDVTINKTEICSQL

(4) 0772P (CA125, MUC16, Genbank accession no. AF361486

J. Biol. Chem. 276 (29):27371-27375 (2001)); WO2004045553 (claim 14); WO200292836 (claim 6; FIG. 12); WO200283866 (claim 15; Page 116-121); US2003124140 (Example 16); US2003091580 (claim 6); WO200206317 (claim 6; Page 400-408);

Cross-references: GI:34501467; AAK74120.3; AF361486_1

6995 aa

(SEQ ID NO: 4)

PVTSLLTPGLVITTD RMGISREPGTSSTSNLSSTSHERLTLEDVTDTTEAMQPSHTAVT
 NVRTSISGHESQSSVLSDSETPKATSPMGTTYTMGETSVSISTSDFFETSRIQIEPTSSL
 TSGLRETSSSERISSATEGSTVLSEVP SGATTEVSRTEVISSRGTSMSGPDQFTISPDIS
 TEAITRLSTSPIMTESAESAITIETGSPGATSEGLTLDTSTTTEWSGTHSTASPGFSHS
 EMTTLMSTRTPGDVPWPSLPSVEEASSVSSSLSPAMTSTSFESTLPESISSPHPV TALL
 TLGPVKTTDMLRTSSEPETSPPNLSSTSAEILATSEVTKDREKIHPSNTPVNVGTVI
 YKHLSPSSVLADLVTTKTPSPMATTSTLGNTSVSTSTPAPPETMMTQPTSSSLTSLGREIS
 TSQETSSATERSASLSGMPTGATTKVSRTEALSLGRTSTPGPAQSTISPEISTETITRIS
 TPLTTTGSAEMTITPKTGHSGASSQGTFTLDTSSRASWPGTHSAATHRSPHSGMTTPMSR
 GPEDVSWPSRPSVEKTSPPSSVLVSLAVTSPSPLYSTPSESSHSSPLRVTSLFTPVMMKT
 TDMLDTSLEPVTTSPSMNITSDES LATSKATMETEAIQLSENTAVTQMGTISARQEFYS
 SYPGLPEPSKVTSPVVTSSTIKDIVSTTIPASSEITRIEMESTSLTPTPRETSTSQEIH

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SATKPSTVPYKALTSATIEDSMTQVMSSSRGSPDQSTMSQDISTEVITRLSTSPIKTES
 TEMTITTQTGSPGATSRGTLTLDSTTFMSGTHSTASQGFHSQMTALMSRTPGEVPWLS
 HPSVEEASSASFSLSSPVMTSSSPVSSSTLPDSIHSSSLPVTSLLTSGLVKTTTELLGTSSE
 PETSSPNLSSSTSAEILATTEVTDTDEKLEMTNVVTSYGYTHESPSSVLADSVTTKATSSM
 GITYPTGDTNVLTSTPAFSDTSRIQTKSKLSLTPGLMETSISEETSSATEKSTVLSVPT
 GATTEVSRTEAIISSRTSIPGPAQSTMSSDTSMETITRISTPLTRKESTDMAITPKTGPS
 GATSQGTFTLDDSSSTASWPGTHSATTQRFPRSVVTPMSRGPEDVSWPSPLSVEKNSPPS
 SLVSSSVTSPSPLYSTPSGSSHSPVPVTSLFTSIMMKATMDLDASLEPETTSAPNMNI
 TSDSLAASKATTETEAHVHFENTAASHVETTSATEELYSSSPGFSEPTKVISPVTSSS
 IRDNMVSTTMPGSSGITRIEIESMSSLTPGLRETRTSQDITSSSTETSTVLYKMPSGATPE
 VSRTEVMPSSRTSIPGPAQSTMSLDDISDEVVTRLSTSPIMTESAEITITTQTGYSLATSQ
 VTLPLGTSMTFLSGTHSTMSQGLSHSEMNLMSRGPELSWTSPRFVETTRSSSLTSLP
 LTTSLSPVSSLLDSSPSSPLPVTSLILPGLVKTTTEVLDTSSEPKTSSPNLSSSTSVIEIP
 ATSEIMTDTEKIHPSNNTAVAKVRTSSSVHESHSSVLADSETTITIPSMGITSAVEDITV
 PTSNPAFSETRRIPTTEPTFSLTPGPRETSTSEETTSITETSAVLFGVPTSATTEVSMTEI
 MSSNRTHIPDSQSTMSPDITTEVITRLSSSSMMSESTQMTITTQKSSPGATAQSTLTLA
 TTTAPLARHSTVPPRFLHSEMTLMSRSPENPSWKSSPFVEKTSSSSSLLSLPVTTSPS
 VSSLTLPQISPSFSVTSLLTGPMVKTDTSTEPGTSLSPNLSGTSVEILAASEVTTDTE
 KIHPSSSMAVTNVGTTSSGHELYSSVSIHSEPSKATYPVGTSPSSMAETSISTSMANFET
 TGFEAEFPFSLTSGLRKTNMSLDTSSVPTNTPTSSPGSTHLLQSSKTDFTSSAKTSSPDW
 PPASQYTEIPVDIITPFNASPSITESTGITSFPESRFTMSVTESTHHLSDLLPSAETIS
 TGTVMPSLSEAMTSFATTGVPRASISGSGSPFSRTESGPGDATLSTIAESLPSSPTVPFSS
 STETTTDSSTIPALHEITSSSATPYRVDTSLGTESSSTEGRLVMVSTLDTSSQPGRSTSS
 PILDTRMTESVELGTVTSAYQVPSLSTRLTRTDGIMEHITKIPNEAAHRTIRPVKGPQT
 STSPASPGLHTGGTKRMETTTTALKTTTALKTTSRATLTTSVYPTLGLTLPNASMQ
 MASTIPTEMMITPYVFPDVPETTSLSLATSGLAETSTALPRTPSVENRESETTASLVSR
 SGAERSPVIQTLDVSSSEPDTTASWVIHPAETIPTVSKTTPNFEHSELDTVSSSTATSHGA
 DVSSAIPTNISPELDAITPLVTISGTDTSSTTEPTLTKSPHETETRTWLTHPAETSSSTI
 PRTPINPFSHHESDATPSIATSPGAETSSAIPIMTVSPGAEDLVTSQVTSSGTRNMTIPT
 LTLSPGEPKTIASLVTHPEAQTSIAIPTSTISPAVSRVLSMVTSLAAKTSTTNRALTNS
 PGEPATTVSLVTHSAQTSPTVPWTTISIFHKSDDTTPSMTTSHGAESSAVPTPTVSTEV
 PGVVTPLVTSRAVISTTIPILTLSPGEPETTPSMATSHGEEASSAIPPTVSPGVPGVV
 TSLVTSSRAVSTTIPILTFSLGEPETTPSMATSHGTEAGSAVPTVLPEVPGMVTSLVAS
 SRAVSTTTLPTLTLSPGEPETTPSMATSHGAEASSTVPTVSPEVPGVVTSVLTSSSGVNS
 TSIPTLILSPGELETTTPMATSHGAEASSAVPTPTVSPGVSGVVTPLVTSSRAVSTTIP
 ILTLSSSEPETTPSMATSHGVEASSAVLTVSPEVPGMVTFVLTSSRAVSTTIPTLTISS
 DEPETTTSLVTHSEAKMISAIPTLGVSPVQGLVTSVLTSSGSETSAFNSLTVASSQPET
 IDSWVAHPGTEASSVPTLTVSTGEPFTNISLVTHPAESSSTLPRTTSRFSHSELDTMP
 TVTSPEAESSSAISTTISPGIPGVLTSLVTSSGRDISATEPTVPESPHSEATASWVTHP
 AVTSTTVPRTPPNYHSEPDTPPSIATSPGAETSDIPTITVSPDVPDMVTSQVTSSGTD
 TSITIPTLTLSSGEPETTTSFITYSETHTSSAIPPLVSPDASKMLTSLVSISSGTDSTTT

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FPTLTETPYEPETTAIQLIHPAETNTMVPRTTPKESHKSDTTLPVAITSPGPEASSAVS
 TTTISPDMSDLVTSLPVSSGTDSTTEPTLSETPYEPETTATWLTHPAETSTTVSGTIPN
 FSHRGSDTAPSMVTS PGVDTRSGVPTTTIPPSIPGVVTSQVTSSATDSTAIPTLTPSPG
 EPETTASSATHPGTQTGFTVPIRTVPSSEPDTMASWVTHPPQTSTPVSRTTSSFSHSSPD
 ATPVMATSPRTEASSAVLTTISPGAPEMVTSQITSSGAATSTTVPTLTHSPGMPETTALL
 STHPRTESTSKTFPASTVFPQVSETTASLTIRPGAETSTALPTQTTSSSLFTLLVTGTSRVD
 LSPTASPGVSAKTAPLS THPGTETSTMIPTSTLSLGLLETTGLLATSSSAETSTSTLTLT
 VSPAVSGLLSSASITTDKPQTVTSWNTETSPSVTSVGPPEFSRTVTGTTMTLIPSEMPTP
 KTSHGEGVSPPTILRTTMVEATNLATTGSSPTVAKTTTTENTLAGSLFTPLTTPGMSTLA
 SESVTSRTSYNHRSWISTSSYNRYWTPATSTPVTSTESPGISTSSIPSSAATVPFMV
 PFTLNFTITNLQYEEDMRHPGSRKFNATERELQGLLKPLFRNSSLEYLYSGCRLASLRPE
 KDSSATAVDAICTHRPDPEDLGLDRERLYWELSNLTNGIQELGPYTLDRNSLYVNGFTHR
 SSMPPTSTPGTSTVDVGTSGTPSSSPSTTAGPLLMPTLNFTITNLQYEEDMRRTGSRK
 FNTMESVLQGLLKPLEKNTSVGPLYSGCRLTLRPEKDGAATGVDAICTHRLDPKSPGLN
 REQLYWELSKLTNDIEELGPYTLDRNSLYVNGFTHQSSVSTSTPGTSTVDLRTSGTPSS
 LSSPTIMAAGPLLVPTLNFTITNLQYGEDMGHPGSRKENTTERVLQGLLGPIEKNTSVG
 PLYSGCRLTSLRSEKDGAATGVDAICHHLDPKSPGLNRERLYWELSQLTNGIKELGPYT
 LDRNSLYVNGFTHRTSVPTTSTPGTSTVDLGTSGTPFSLPSPATAGPLLVFTLNFTITN
 LKYEEDMRHPGSRKFNTTERVLQTLVGPMFKNTSVGLLYSGCRLTLRSEKDGAATGVDA
 ICTHRLDPKSPGVDRQLYWELSQLTNGIKELGPYTLDRNSLYVNGFTHWIPVPTSSTPG
 TSTVDLGTSGTPSSLPSPSATAGPLLVPTLNFTITNLKYEEDMHCPGSRKENTTERVLQ
 SLLGPMKNTSVGPLYSGCRLTLRSEKDGAATGVDAICTHRLDPKSPGVDRQLYWELSQL
 TNGIKELGPYTLDRNSLYVNGFTHQTSAPNTSTPGTSTVDLGTSGTPSSLPSPSATAGP
 LLVPFTLNFTITNLQYEEDMRHPGSRKENTTERVLQGLLGPMKNTSVGLLYSGCRLTLR
 RPEKNGAATGMDAICSHRLDPKSPGLNRQLYWELSQLTHGIKELGPYTLDRNSLYVNGF
 THRSSVAPTSTPGTSTVDLGTSGTPSSLPSPSTAVPLLVPTLNFTITNLQYGEDMRHPG
 SRKFNTTERVLQGLLGPLFKNSSVGPLYSGCRLISLRSEKDGAATGVDAICTHHLNPQSP
 GLDREQLYWQLSQMTNGIKELGPYTLDRNSLYVNGFTHRSSGLTTSTPWTSTVDLGTSGT
 PSPVPSPTTAGPLLVPTLNFTITNLQYEEDMRHPGSRKFNATERVLQGLLSPIFKNSSV
 GPLYSGCRLTSLRPEKDGAATGMDAVCLYHPNPKRPGLDREQLYWELSQLTHNITELGPY
 SLDRDSLYVNGFTHQNSVPTTSTPGTSTVYWATTGTPSSFPGHTEPGPLLIPTFNTIT
 NLHYEENMQHPGSRKFNTTERVLQGLLKPLFKNTSVGPLYSGCRLTLRPEKQEAATGVD
 TICTHRVDPIGPGLDRERLYWELSQLTNSITELGPYTLDRDSLYVNGENPWSSVPTTSTP
 GTSTVHLATSGTPSSLPGHTAPVPLLIPTLNFTITNLHYEENMQHPGSRKENTTERVLQ
 GLLKPLEKSTSVGPLYSGCRLTLRPEKHGAATGVDAICTRLDPTGPGLDREERLYWELSQL
 TNSVTTELGPYTLDRDSLYVNGFTHRSSVPTTSTPGTSAVHLETSGTPASLPGHTAPGP
 LLVPFTLNFTITNLQYEEDMRHPGSRKENTTERVLQGLLKPLEKSTSVGPLYSGCRLTLR
 RPEKNGAATGVDTICTHRLDPLNPGLDREQLYWELSKLTRGIIELGPYLLDRGSLYVNGF
 THRNEVPISTPGTSTVHLGTSETPSSLPRIVPGPLLVPTLNFTITNLQYEEAMRHPG
 SRKENTTERVLQGLLRPLFKNTSIGPLYSSCRLTLRPEKDKAATRVDAICTHHPDPQSP
 GLNREQLYWELSQLTHGITELGPYTLDRDSLYVDGFTHWSPIPTTSTPGTSTIVNLGTSGI

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PPSLPETTATGPLLVPFTLNFTITNLQYEENMGHPGSRKFNITESVLQGLLKPLEKSTSV
 GPLYSGCRLTLRLPEKDGVAIVDAICTHRPDKIPGLDRQQLYWELSQLTHSITELGPY
 TLDRLSLYVNGFTQRRSSVPTTSTPGTFTVQPETSETPSSLPGPATGTVLLPFTLNFTII
 NLQYEEDMHRPGSRKFNTERVLQGLLMPLFKNTSVSSLYSGCRLTLRLPEKDGAAITRD
 AVCTHRPDKSPGLDRERLYWKLSQLTHGITELGPYTLDRHSLYVNGFTHQSSMTTTRTP
 DTSTMLATSRTPASLSGPTTASPLLVFTINFTITNLRYEENMHHPGSRKFNTERVLQ
 GLLRPVFKNTSVGPLYSGCRLTLRLPKKDGAATKVDAICTYRPDKSPGLDREQLYWELS
 QLTHSITELGPYTLDRSLYVNGFTQRRSSVPTTSIPGTPTVDLGTSGTPVSKPGPSAASP
 LLVLFTLNFTITNLRYEENMHHPGSRKFNTERVLQGLLSLEKSTSVGPLYSGCRLTLRL
 RPEKDGATGVDAICTHHPDKSPRLDREQLYWELSQLTHNITELGPYALDNDLSLEVNGF
 THRSSVSTTSTPGTPTVYLGAASKTPASIFGPSAASHLLILFTLNFTITNLRYEENMWPGS
 RKFNTTERVLQGLLRPLFKNTSVGPLYSGCRLTLRLPEKDGATGVDAICTHRPDKTPGPG
 LDREQLYLELSQLTHSITELGPYTLDRSLYVNGFTHRSSVPTTSTGVVSEEPFTLNFTI
 NNLRYMADMGQPGSLKFNI TDNVMQHLSPFQRRSSLGARYTGCRVIALRSVKNGAETRV
 DLLCTYLQPLSGPLPIKQVFHELSSQTHGITRLGPYSLDKDSLYLNGYNEPGDEPPTT
 PKPATTFLPLSEATTAMGYHLKTLTNFTISNLQYSPDMGKSATFNSTEGVLQHLRLP
 LPQKSSMGPFYLGQCLISLRPEKDGAAITGVDTTCTYHPDPVGPGLDIQQLYWELSQLTHG
 VTQLGFYVLDRLSLFINGYAPQNLISIRGEYQINFHIVNWNLSNPDPSTSEYITLLRDIQD
 KVTTLTKGSQLHDTFRFCLVTNLTMDSVLVTVKALFSSNLDPSLVEQVFLDKTLNASPHW
 LGSTYQLVDIHVTEMESSVYQPTSSSSSTQHLYFNFTITNLPYSQDKAQPGTTNYQRNKRN
 IEDALNQLFRNSSISIKYFSDCQVSTFRSVPNRHHTGVDSL CNFSPLARRVDRVAIYEEFL
 RMRNGTQLQNTLDRSSSVLDGYSFNRNEPLTGNSDLPFWAVILIGLAGLLGLITCLIC
 GVLVTTRRRKKEGEYVNVQQCPGYQSHLDLEDLQ

(5) MPF (MPF, MSLN, SMR, megakaryocyte potentiating factor, mesothelin, Genbank accession no. NM_005823

Yamaguchi, N., et al. Biol. Chem. 269 (2), 805-808 (1994), Proc. Natl. Acad. Sci. USA. 96 (20):11531-11536 (1999), Proc. Natl. Acad. Sci. USA. 93 (1):136-140 (1996), J. Biol. Chem. 270 (37):21984-21990 (1995); WO2003101283 (claim 14); (WO2002102235 (claim 13; Page 287-288); WO2002101075 (claim 4; Page 308-309); WO200271928 (Page 320-321); WO9410312 (Page 52-57);

Cross-references: MIM:601051; NP_005814.2; NM_005823_1

622 aa

(SEQ ID NO: 5)

MALPTARPLLGSCTPALGSLFLFSLGWVQPSRTLAGETGQEAAPLDG
 VLANPPNISSLSRQLLGFPCAIEVSGSLSTERVRELAVALAQKNVKLSTEQ
 LRCLAHRLSEPPEDLDALPLDLLFLNPDAFSGPQACTRFFSRITKANVD
 LLPRGAPERQRLLPAAALACGWGRSLLSEADVRLGLGLACDLPRFVAES
 AEVLLPRLVSCPGPLDQDQQAARAALQGGPPYGPSTWSVSTMDALRG
 LLPVLGQPIIRSIPQIVAAWRQSSRDPSWRQPRTILRPRFRREVEKT
 ACPSGKKAREIDESLIFYKKWELEACVDAALLATQMDRVNAIPFTYEQLD
 VLKHKLEDELYPQGYPSVQHLGYLFLKMSPEDIRKWNVTSLKLEKALLE

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VNKGHEMSPQVATLIDRFVKGGRQDLKDTLDTLTAFYPGYLCSLSPEELS
 SVPPSSIWAVRPQDLDTCDPRQLDVLYPKARLAFQNMNGSEYFVKIQSFL
 GGAPTEDLKALSQQNVSMDLATFMKLRTDAVLPLTVAEVQKLLGPHVEGL
 KAEERHRPVRDWILRQRQDDLTGLGLQGGIPNGYLVLDLSMQEALSGT
 PCLLGGPGVLTVALLLASTLA

(6) Napi3b (NAPI-3B, NPTIIB, SLC34A2, solute carrier family 34 (sodium phosphate), member 2, type II sodium-dependent phosphate transporter 3b, Genbank accession no. NM_006424, J. Biol. Chem. 277 (22):19665-19672 (2002), Genomics 62 (2):281-284 (1999), Feild, J. A., et al. (1999) Biochem. Biophys. Res. Commun. 258 (3):578-582; WO2004022778 (claim 2); EP1394274 (Example 11); WO2002102235 (claim 13; Page 326); EP875569 (claim 1; Page 17-19); WO200157188 (claim 20; Page 329); WO2004032842 (Example IV); WO200175177 (claim 24; Page 139-140);

Cross-references: MIM:604217; NP_006415.1; NM_006424_1

690 aa

(SEQ ID NO: 6)

MAPWPELGDAQPNPDKYLEGAAGQQPIAPDKSKEINKTDNTEAPVTKIEL
 LPSYSTATLIDEPTEVDDPWNLPQLQDSGIKWSERDTKGKILCFQGGIGR

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LILLGLFLYFFVCSLDILSSAFQLVGGKMAQFFSNSSIMSNPLLGLVIG
VLVTVLVQSSSISTSIIVSMVSSSLIVRAAPIIMGANIGTSITNTIVA
LMQVGDRESEFFRAFAGATVHDFFNWLSVLVLLPVEVATHYLEIITQLIVE
SPHFKNGEDAPDLLKVITKPFKTLIVQLDKKVISQIAMNDEKAKNSLVK
IWCKTFINKTQINIVIPSTANCTSPSLCWIDGIQNWTKNVTYKENIAKC
QHIFVNFHLPDLAVGTILLILSLVLCGLIMIVKILGSLVKQVATVIK
KTINTDPPFPFAWLIGYLAILVAGMTFIVQSSSVFTSALTPLIGIGVIT
IERAYPLTLGNSNIGTITTAILAALASPGNALRSSQLALCHFFFNISGIL
LWYPIPFTRLPIRMAKGLGNISAKYRFAVFYLIIFFLIPLIVFGLSLA
GWRVLVGVGVVVFIIILVLCRLQLQSRCPRVLPPKLLQNWNLPLWMSL
KPWDAVSVKFTGCFQMRCCYCCRVCCRACLLCGCPKCCRCSCCEDLEE
AQEGQDVPVKAPETFDNITISREAQGEVPASDSKTECTAL

(7) Sema 5b (F1110372, KIAA1445, Mm.42015, SEMA5B, SEMAG, Semaphorin 5b Hlog, sema domain, seven thrombospondin repeats (type 1 and type 1-like), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 5B, Genbank accession no. AB040878, Nagase T., et al. (2000) DNA Res. 7 (2):143-150; WO2004000997 (claim 1); WO2003003984 (claim 1); WO200206339 (claim 1; Page 50); WO200188133 (claim 1; Page 41-43, 48-58); WO2003054152 (claim 20); WO2003101400 (claim 11);

Accession: Q9P283; EMBL; AB040878; BAA95969.1. Genew; HGNC:10737;

1093 aa (SEQ ID NO: 7)
MVLAGPLAVSLLLPSLTLVSHLSSSQDVSSEPSSEQQLCALSKHPTVAF
EDLQPWVSNFTYPGARDFSQLALDPSGNQLIVGARNYLFRSLANVSLQ
ATEWASSEDTRRSQSGKTEEBEQNYVRVLIAGRKVFMCGTNAFSPMC
TSRQVGNLSRTTEKINGVARCPYDPRHNSTAVISSQGEIYAATVIDFSGR
DPAIYRSLGSGPPLRTAQYNSKWLNEPNEVAAYDIGLFAYFFLRENAVEH
DCGRTVYSRVARVCKNDVGGRELLEDWTTFMKAHLNCSRPGVEVPFYNE
LQSAFHLPEQDLIYGVTETNVNSIAASAVCAFNLSAISQAFNGPFRYQEN
PRAAWLPANIPINPFCGTLPETGPNENLTERSLQDAQRLFLMSEAVQP
TPEPCVTQDSVRFSHLVVDLVQAKDTLYHVLYIGTESGTLKALSTASRS
LHGCYLEELHVLPPGRREPLRLSLRILHSARALFVGLRDGVLRVPLERCAA
YRSQGAACLGARDPYCGWDGKQRCSTLEDSSNMSLWTQNTACPVNRVTR
DGGFGPWSWPQCEHLDGDNSSGCLCRARS CDSRPRCGGLDCLGPAIHI
ANCSRNGAWTPWSSWALCSTSCGIGFQVRQSRCSNPAPRHGGRICVGSKR
EERFCNENTPCPVPIFWASGWSKCSNCGGMQSRRRACENGNSCLGC
GVEFKTCNPEGCEVRRNTPTWPLVNVNTQGGARQEQRFRTCRAPLAD
PHGLQFRRRTETRTCPADGSGSCD DALVEDLLRSGSTSPHTVSGGWAA
WGPWSSCSRDCELGFRVRKRTCTNPEPRNGGLPCVGDAEYQDCNPQACP
VRGAWSWTWSPCSASCGGGHYQRTRCTSPAPSPGEDI CLGLHTEEAL
CATQACPEGWSPWSEWSKCTDDGAQSRSRHCEELLPGSSACAGNSSQSRP

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CPYSEIPVILPASSMEEATGCAGFNLIHLVATGISCFLGSGLLTAVYLS
CQHCQRQSQESTLVHPATPNHLHYKGGTTPKNEKYTPMEFKTLNKNLIP

5 DDRANFYPLQQTNVYTTTYPSPLNKHSFRPEASPGQRCFPNS
(8) PSCA hlg (2700050C12Rik, C₅₃₀₀₀₈O16Rik, RIKEN cDNA 2700050C12, RIKEN cDNA 2700050C12 gene, Genbank accession no. AY358628); US2003129192 (claim 2); US2004044180 (claim 12); US2004044179 (claim 11); US2003096961 (claim 11); US2003232056 (Example 5); WO2003105758 (claim 12); US2003206918 (Example 5); EP1347046 (claim 1); WO2003025148 (claim 20);

Cross-references: GI:37182378; AAQ88991.1;
15 AY358628_1

141 aa (SEQ ID NO: 8)
MWVLGIAATFCGLFLLPGFALQIQCYQCEEFQLNNDCSSPEFIVNCTNVV
20 QDMCQKEVMEQSAGIMYRKSCASSAACLIASAGYQSFCSPGKLSNVCISC
CNTPLCNGPRPKRGSSASALRPGLRTTILFLKLALFSAHC

(9) ETBR (Endothelin type B receptor, Genbank accession no. AY275463);

Nakamuta M., et al. Biochem. Biophys. Res. Commun. 177, 34-39, 1991; Ogawa Y., et al. Biochem. Biophys. Res. Commun. 178, 248-255, 1991; Arai H., et al. Jpn. Circ. J. 56, 1303-1307, 1992; Arai H., et al. J. Biol. Chem. 268, 3463-3470, 1993; Sakamoto A., Yanagisawa M., et al. Biochem. Biophys. Res. Commun. 178, 656-663, 1991; Elshourbagy N. A., et al. J. Biol. Chem. 268, 3873-3879, 1993; Haendler B., et al. J. Cardiovasc. Pharmacol. 20, S4-S4, 1992; Tsutsumi M., et al. Gene 228, 43-49, 1999; Strausberg R. L., et al. Proc. Natl. Acad. Sci. USA. 99, 16899-16903, 2002; Bourgeois C., et al. J. Clin. Endocrinol. Metab. 82, 3116-3123, 1997; Okamoto Y., et al. Biol. Chem. 272, 21589-21596, 1997; Verheij J. B., et al. Am. J. Med. Genet. 108, 223-225, 2002; Hofstra R. M. W., et al. Eur. J. Hum. Genet. 5, 180-185, 1997; Puffenberger E. G., et al. Cell 79, 1257-1266, 1994; Attie T., et al. Hum. Mol. Genet. 4, 2407-2409, 1995; Auricchio A., et al. Hum. Mol. Genet. 5:351-354, 1996; Amiel J., et al. Hum. Mol. Genet. 5, 355-357, 1996; Hofstra R. M. W., et al. Nat. Genet. 12, 445-447, 1996; Svensson P. J., et al. Hum. Genet. 103, 145-148, 1998; Fuchs S., et al. Mol. Med. 7, 115-124, 2001; Pingault V., et al. (2002) Hum. Genet. 111, 198-206; WO2004045516 (claim 1); WO2004048938 (Example 2); WO2004040000 (claim 151); WO2003087768 (claim 1); WO2003016475 (claim 1); WO2003016475 (claim 1); WO200261087 (FIG. 1); WO2003016494 (FIG. 6); WO2003025138 (claim 12; Page 144); WO200198351 (claim 1; Page 124-125); EP522868 (claim 8; FIG. 2); WO200177172 (claim 1; Page 297-299); US2003109676; U.S. Pat. No. 6,518,404 (FIG. 3); U.S. Pat. No. 5,773,223 (claim 1a; Col 31-34); WO2004001004;

442 aa (SEQ ID NO: 9)
60 MQPPPSLCGRALVALVLACGLSRIWGEERGFPDPDRATPLQTAETMPTT
KILWPKGSNASLARS LAPAEVPGDRTAGSPPTISPPPCQGPPIKETF
KYINTVVSCLVFLVLIIGNSTLLRIYKMKMRNGPNILIASLALGDLH
IVIDIPINVYKLLAEDWPFGAEMCKLVFFIQKASVGITVLSLCAISIDRY
65 RAVASWSRIKIGIVPKWTAVEIVLIWVSVVLAVPEAIGFDIITMDYKGS

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YLRICLLHPVQKTAQMIFYKTAKDWLFSFYFCLPLAITAFFYILMICEM
LRKKSQMIALNDHLKQRREVAKTVFCLVLVFCWLPPLHLSRILKLTLY
NQNDPNRCELLSFLLLVDYIGINMASLNSCINPIALYLVSKRFKNCFKSC
LCCWCQSFEEKQSLEEKQSCCLKPKANDHGYDNFRSSNKYSSS

(10) MSG783 (RNF124, hypothetical protein F1120315, Genbank accession no. NM_017763); WO2003104275 (claim 1); WO2004046342 (Example 2); WO2003042661 (claim 12); WO2003083074 (claim 14; Page 61); WO2003018621 (claim 1); WO2003024392 (claim 2; FIG. 93); WO200166689 (Example 6);

Cross-references: LocusID:54894; NP_060233.2; NM_017763_1

783 aa

(SEQ ID NO: 10)

MSGGHQLQLAALWPWLLMATLQAGFRTGLVLA AAVESERSAEQKAIIRV
IPLKMDPTGKLNLTLEGVFAGVAEITPAEGKLMQSHPLYLCNASDDDNLE
PGFISIVKLESPPRAPRPCLSLASKARMAGERGASAVLPDITEDRAAAEQ
LQQPLGLTWPVVLWIWNGDAEKLEMFVYKNQKAHVRIELKEPPAWPDYDVW
ILMTVVGTIFVILASVLRIRCRPRHSRPDPLQORTAWAISQLATRRYQA
SCRQARGEPDSSGSSSAPVCAICLEEFSEGQELRVISCLHEFHRCNVD
PWLHQHRTCLCVFNITEGDSFSQSLGSPRSYQEPGRRLHLIRQHPGHAH
YHLPAAYLLGSPRSASAVARPPRPGPFLPSQEPGMGPRHRRFPRAAHPRAPG
EQQRLAGAQHPYAQGWGMSHLQSTSQHPAACPVPLRRARPDSGSGESY
CTERSGYLADGPASDSSSGPCHGSSSDSVVNCTDISLQGVHGSSTFCSS
LSSDFDPLVYCSPKGPQVRVDMQPSVTSRPRSLDSVVPGETQVSSHVHY
HRHRHHHYKRFQWHGRKPGPETGVPQSRPPIPRTPQPEPPSPDQQVTG
SNSAAPSGRSLSNPQCPRALPEPAGPVDASSICPSTSSLPNLQKSSLSAR
HPQRKRRGGPSEPTPGSRPQDATVHPACQIFPHYTPSVAYPWSPEAHPLI
CGPPGLDKRLLPETPGPCYSNSQPVWLCLTPRQPLEPHPPGEGPSEWSSD
TAEGRPCYPHQCQVLSAQPGSEEELEELCEQAV

(11) STEAP2 (HGNC_8639, IPCA-1, PCANAP1, STAMP1, STEAP2, STMP, prostate cancer associated gene 1, prostate cancer associated protein 1, six transmembrane epithelial antigen of prostate 2, six transmembrane prostate protein, Genbank accession no. AF455138, Lab. Invest. 82 (11):1573-1582 (2002)); WO2003087306; US2003064397 (claim 1; FIG. 1); WO200272596 (claim 13; Page 54-55); WO200172962 (claim 1; FIG. 4B); WO2003104270 (claim 11); WO2003104270 (claim 16); US2004005598 (claim 22); WO2003042661 (claim 12); US2003060612 (claim 12; FIG. 10); WO200226822 (claim 23; FIG. 2); WO200216429 (claim 12; FIG. 10);

Cross-references: GI:22655488; AAN04080.1; AF455138_1

490 aa

(SEQ ID NO: 11)

MESISMMGSPKLSSETVLPNGINGIKDARKVIVGVIGSDPAKSLTIRLI
RCGYHVIVIGSRNPKFASEFPFHVVDVIHHEDALTINIIFVAIHREHYTS
LWDLRHLHVGKILIDVSNMNRINQYPESNAEYLASLFPDSLIVKGFNVVS

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AWALQLGPKDASRQVYICSNNIQARQQVIELARQLNFIPIDLGLSSSARE
IENLPLRLFTLWRGPVVVAISLATFFFLYSFVRDVIHPYARNQQSDFYKI
PIEIVNKTLPVITAITLLSLVYLAGLLAAAYQLYYGTKYRRFPFWLETWLQ
CRKQLGLLSFFFAMVHVAYSCLCLPMRRSERYFLNLMAYQQVHANIENSWN
EEEVWRIEMYISFGIMSLGLLSLLAVISIPSVSNALNWREFSFIQSTLGY
VALLISTFHVLIYGWKRAFEYEEYRFYIPPNFVLALVLPSPVILGKIILF
LPCISQKLKRIKKGWEKSQFLEEGIGGTIPHVSPERVTVM

(12) TrpM4 (BR22450, FLJ20041, TRPM4, TRPM4B, transient receptor potential cation channel, subfamily M, member 4, Genbank accession no. NM_017636 Xu, X. Z., et al. Proc. Natl. Acad. Sci. USA. 98 (19):10692-10697 (2001), Cell 109 (3):397-407 (2002), J. Biol. Chem. 278 (33):30813-30820 (2003)); US2003143557 (claim 4); WO200040614 (claim 14; Page 100-103); WO200210382 (claim 1; FIG. 9A); WO2003042661 (claim 12); WO200230268 (claim 27; Page 391); US2003219806 (claim 4); WO200162794 (claim 14; FIG. 1A-D);

Cross-references: MIM:606936; NP_060106.2; NM_017636_1

1214 aa

(SEQ ID NO: 12)

MVVPEKEQSWIPKIFKKKTCTTFIVDSTDPGGTLCCQGRPRTAHPAVAME
DAFGAAVTVWSDAHTTEKPTDAYGELDTAGRKHNSFLRLSDRTDPA
AVYSLVTRTWGFRAPNLVSVLGGSGGPVLQTLWQDLLRRGLVRAAQSTG
AWIVTGLLHTGIGRHVGVAVRDHQMSTGGTKVVMGAPWGVVRNRDTL
INPKGSFPARYRWGDPEDGVQFPLDYNYSAPFLVDDGTHGCLGGENRFR
LRLESYISQKQTKVGGTGIDIPVLLLLIDGDEKMLTRIENATQAQLPCLL
VAGSGGAADCLAETLEDTLAPGSGGARQGEARDIRRRFPFGKDEVLQAQ
VERIMTRKELLTVYSSSEDGSEEFETIVLKALVKACSSSEASAYLDELRLA
VAVNRVDIAQSELFRGDIQWRSFHLASLMDALLNDRPEFVRLLI SHGLS
LGHFLTPMRLAQLYSAAPSNLSLRNLLDQASHSAGTKAPALKGGAELRP
PDVGHVLRMLLGKMCAPRYPSGGAWDPHPGQGFGESEMYLLSDKATSPLSL
DAGLGQAPWSDLLWALLLNRAQMAMYFWEMGSNAVSSALGACLLLRVMA
RLEPDAAEEAARRKDLAFKPEGMGVDLFGECYRSSEVRAARLLLRRCPLWG
DATCLQLAMQADARAFFAQDGVQSLLTQKWWGDMASTTPIWALVLAFFCP
PLIYTRLIITPRKSEEEPTREELEFDMDSVINGEGPVGTADPAEKTPLGVP
RQSGRPGCCGRCGRRCLRRWFHFWGAPVTIFMGNVVSYLLFLLLF SRV
LLVDFQPAPPGLLELLLYFWAFILLCEELRQGLSGGGGSLASGGPGPGHA
SLSQRLRLYLADSWNQCDLVALTCFLLGVGCRILPGLYHLGRIVLCIDFM
VFIVRLHIFTVNKQLGPKIVIVSKMMKDVFFLFLFGLVWLVAYGVATEG
LLRPRDSDFPSILRRVYRYPYLIQIFGQIPQEDMDVALMEHNSCSSEPGFW
AHPPGAQAGICVSQYANWLVLVLLVIFLLVANILLVNLIIAMFSYTFGKV
QGNSDLYWKAQRYRLIREFHSRPAAPPFIVISHLRLRLQLCRRPRSPQ
PSSPALEHFRVYLSKEAERKLLTWESVHKENFLARARDKRESDSERLKR

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ISQKVDLALKQLGHIREYEQRLKVLEREVQQCSRVLGWVAEALSRSALLP
PGGPPPPDLPGSKD

(13) CRIPTO (CR, CR1, CRGE, CRIPTO, TDGF1, 5
teratocarcinoma-derived growth factor, Genbank accession
no. NP_003203 or NM_003212,

Ciccociola, A., et al. EMBO J. 8 (7):1987-1991 (1989),
Am. J. Hum. Genet. 49 (3):555-565 (1991)); US2003224411
(claim 1); WO2003083041 (Example 1); WO2003034984
(claim 12); WO200288170 (claim 2; Page 52-53);
WO2003024392 (claim 2; FIG. 58); WO200216413 (claim
1; Page 94-95, 105); WO200222808 (claim 2; FIG. 1); U.S.
Pat. No. 5,854,399 (Example 2; Col 17-18); U.S. Pat. No.
5,792,616 (FIG. 2);

Cross-references: MIM:187395; NP_003203.1;
NM_003212_1

188 aa

(SEQ ID NO: 13)

MDCRKMARFYSYVIWIMAIKVFELGLVAGLGHQEFARPSRGYLAFRDDSD
IWPQEEPAIRPRSSQRPVPMGIQHSKELNRIICLNGGICMLGSFCACPPS
FYGRNCEHDVRKENCQSVPHDTWLPKKCSLCKCWHGQLRCFPQAFPLPGCD
GLVMDHLVASRIPELPPSARITTFMLVGICLSIQSY

(14) CD21 (CR₂ (Complement receptor 2) or C3DR
(C3d/Epstein Barr virus receptor) or Hs.73792 Genbank
accession no. M26004,

Fujisaku et al. (1989) J. Biol. Chem. 264 (4):2118-2125);
Weis J. J., et al. J. Exp. Med. 167, 1047-1066, 1988; Moore
M., et al. Proc. Natl. Acad. Sci. USA. 84, 9194-9198, 1987;
Barel M., et al. Mol. Immunol. 35, 1025-1031, 1998; Weis
J. J., et al. Proc. Natl. Acad. Sci. USA. 83, 5639-5643, 1986;
Sinha S. K., et al. (1993) J. Immunol. 150, 5311-5320;
WO2004045520 (Example 4); US2004005538 (Example 1);
WO2003062401 (claim 9); WO2004045520 (Example 4);
WO9102536 (FIGS. 9.1-9.9); WO2004020595 (claim 1);

Accession: P20023; Q13866; Q14212; EMBL; M26004;
AAA35786.1.

1033 aa

(SEQ ID NO: 14)

MGAAGLLGVFLALVAPGVLGISGSPPPILNGRISYSTPIAVGTVIRYS
CSGTFRLLIGEKSLLCITKDKVDGTWDPAPKCEYFNKYSSCPEPIVPGGY
KIRGSTPYRHGDSVTFACKTNFSMNGKSVWCQANNMNGPTRLPTCVSVF
PLECPALPMIHNGHHTSENVSIAPLSVTYSCESGYLLVGEKIINCLSS
GKWSAVPPTCEEARCKSLGRFPNGKVKEPPILRVGTANFFCDEGYRLQG
PPSSRCVIAGQGVAWTKMPVCEEIFCPSPPPILNGRHIGNSLANVSYGSI
VYTCDDPDPEBGNFIFILIGESTLRCCTVDSQKTGTWSGPAPRCLESTSAVQ
CPHPQILRGRMVSGQKDRYTYNDTVIFACMFGFTLKGSQKIRCAQGTWE
PSAPVCEKECQAPPNINLQKEDRHMVRDPGTSIKYSCNPGYVLVGEES
IQCTSEGVTWPPVPQCKVAACEATGRQLLTKPQHGFVRPDVNSSCGEGYK
LSGSVYQECQGTIPWFMEIRLKEITCPTPPVINYAHTGSSLEDFPYGT
TVTYTCNPGPERGVEFSLIGESTIRCTSNQERGTWSGPAPLCKLSLLAV
QCSHVHIANGYKISGKEAPFYNDTVTFKCYSGFTLKGSSQIRCKADNTW

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DPEIPVCEKETCQHVRQSLQELPAGSRVELVNTSCQDGYQLTGHAYQMCQ
DAENGIWFKKIPLCKVIHCHPPPVIIVNGKHTGMAENFLYGNEVSEYCDQ

GFYLLGEKLLQCRSDSKGHGSWSGSPQCLRSPPVTRCPNPEVKHGYKLN
KTHSAYSHNDIVYVDCNPGFIMNGSRVIRCHTDNTWVPGVPTCIKKAFIG
CPPPPKTPNGNHTGGNIARFSPGMSILYSCDQGYLLVGEALLCTHEGTW
10 SQPAPHCKEVCNCSPPADMGIQKLEPRKMYQYGAVVTLECEDGYMLEGS
PQSQQCSQSDHQWNPPLAVCRSLAPVLCGIAAGLILTLFLIVITLYVISK
HRERNYTTDTSQKEAFHLEAREVSVDPYNPAS

(15) CD79b (CD79B, CD79 β , Igb (immunoglobulin-
associated beta), B29, Genbank accession no. NM_000626
or 11038674, Proc. Natl. Acad. Sci. USA. (2003) 100
(7):4126-4131, Blood (2002) 100 (9):3068-3076, Muller et
al. (1992) Eur. J. Immunol. 22 (6):1621-1625);
20 WO2004016225 (claim 2, FIG. 140); WO2003087768,
US2004101874 (claim 1, page 102); WO2003062401 (claim
9); WO200278524 (Example 2); US2002150573 (claim 5,
page 15); U.S. Pat. No. 5,644,033; WO2003048202 (claim
1, pages 306 and 309); WO 99/558658, U.S. Pat. No.
25 6,534,482 (claim 13, FIG. 17A/B); WO200055351 (claim
11, pages 1145-1146);

Cross-references: MIM:147245; NP_000617.1.
NM_000626_1

229 aa

(SEQ ID NO: 15)

MARLALSPVPSHWMVALLLLSAEPVPAARSEDYRNPKGSACSRIWQSP
RPIARKRGFTVKMHCMNSASGNVSWLWKQEMDENPQQLKLEKGRMEESQ
35 NESLATLTIIQGIREFDNGIYFCQQKCNNTSEVYQGCGETELRVMGFSTLAQ
LKQRNTLKDGIIMIQTLLIILFIIVPIFLLLDKDDSKAGMEEDHIYEGLD
IDQTATYEDIVILRTGEVKWSVGEHPGQE

(16) FeRH2 (IFGP4, IRTA4, SPAP1A (SH2 domain con-
taining phosphatase anchor protein 1a), SPAP1B, SPAP1C,
Genbank accession no. NM_030764, Genome Res. 13 (10):
2265-2270 (2003), Immunogenetics 54 (2):87-95 (2002),
45 Blood 99 (8):2662-2669 (2002), Proc. Natl. Acad. Sci. USA.
98 (17):9772-9777 (2001), Xu, M. J., et al. (2001) Biochem.
Biophys. Res. Commun. 280 (3):768-775; WO2004016225
(claim 2); WO2003077836; WO200138490 (claim 5; FIG.
18D-1-18D-2); WO2003097803 (claim 12);
50 WO2003089624 (claim 25);

Cross-references: MIM:606509; NP_110391.2;
NM_030764_1

55 508 aa

(SEQ ID NO: 16)

MLLWSLLVIFDAVTEQADSLTLVAPSSVFEGDSIVLKQCGEQNWIKQMA
YHKDNKELSVFKFSDFLIQSAVLSDSGNYFCSTKGQLFLWDKTSNIVKI
60 KVQELFQRPVLTASSFQPIEGGPVSLKCETRLSPQRLDVQLQCFPRENQ
VLGSGWSSSPQLQISAVWSEDGYSWCKAETVTHIRKQSLQSQIHVQRI
PISNVSLERAPGGQVTEGQKLIILCSVAGGTGNVTFSWYREATGTSMGK
KTQRSLSAELEIPAVKESDAGKYCRADNGHVPIQSKVNIIPVIRPVSRLP
65 VLTLSRPGAQAAVGDLLLEHCEALRGSPPILYQFYHEDVTLGNSAPS

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GASFNLSTAEHSGNYSCEANGLGAQCSEAVPVSIISGPDGYRRDLMTAG
VLWGLFGVLGFTGVALLLYALPHKISGESSATNEPRGASRPNPQERTYSS
PTPDMEELQPVYVNVGSDVDVYVSQVWSMQQPESANIRTLLNKDSQV
IYSSVKKS

(17) HER2 (ErbB2, Genbank accession no. M11730, Coussens L., et al. *Science* (1985) 230(4730):1132-1139); Yamamoto T., et al. *Nature* 319, 230-234, 1986; Semba K., et al. *Proc. Natl. Acad. Sci. USA.* 82, 6497-6501, 1985; Swiercz J. M., et al. *J. Cell Biol.* 165, 869-880, 2004; Kuhns J. J., et al. *J. Biol. Chem.* 274, 36422-36427, 1999; Cho H.-S., et al. *Nature* 421, 756-760, 2003; Ehsani A., et al. (1993) *Genomics* 15, 426-429; WO2004048938 (Example 2); WO2004027049 (FIG. 11); WO2004009622; WO2003081210; WO2003089904 (claim 9); WO2003016475 (claim 1); US2003118592; WO2003008537 (claim 1); WO2003055439 (claim 29; FIG. 1A-B); WO2003025228 (claim 37; FIG. 5C); WO200222636 (Example 13; Page 95-107); WO200212341 (claim 68; FIG. 7); WO200213847 (Page 71-74); WO200214503 (Page 114-117); WO200153463 (claim 2; Page 41-46); WO200141787 (Page 15); WO200044899 (claim 52; FIG. 7); WO200020579 (claim 3; FIG. 2); U.S. Pat. No. 5,869,445 (claim 3; Col 31-38); WO9630514 (claim 2; Page 56-61); EP1439393 (claim 7); WO2004043361 (claim 7); WO2004022709; WO200100244 (Example 3; FIG. 4);

Accession: P04626; EMBL; M11767; AAA35808.1. EMBL; M11761; AAA35808.1.

1255 aa (SEQ ID NO: 17)
MELAAALCRWGLLLALLPPGAASTQVCTGTDMLRLPASPEHLDMRLHLY
QGCQVQGNLELTYPNLSLFLQDIQEVQGYVLIHNNQVRQVPLQLRLR
IVRGTLQFEDNYALAVLDNGDPLNNTTPVTGASPGGLRELQLRSLTEILK
GGVLIQRNPQLCYQDTILWKDIFHKNNQLALTIDTNRSRACHPCSPMCK
GSRGWGESSEDCQSLTRTVCCAGCARCKGPLEPTCCHEQCAAGCTGPKHS
DCLACLHFHNSGICELHCPALVTYNTDTFESMPNPEGRYTFGASCVTACP
YNYLSTDVGSCTLVCPHNLQEVTAEDGTQRCCKSKPCARVCYGLGMEHL
REVRAVTSANIQEFAGCKIFGSLAFLPESFDGDPASNTAPLQPEQLQVF
ETLEEITGYLYISAWPDSLPLDSVFNQLQVIRGRILHNGAYSLTLQGLGI
SWLGLRSLRELGSGLALIHNNHLCFVHTVPWDQLFRNPHQALLHTANRP
EDECVGEGLACHQLCARGHCWGPQTQCVNCSQPLRGQECVEECRVLQGL
PREYVNAHRLCPCHPECQPNQSVTCFGPEADQCVACAHYKDPFVCVARC
PSGVKPDLSYMPIWKFPDEBAGQPCPINCTHSCVDLDDKGCPAEQRAS
LTSIIISAVVGILLVVVLGVVEGILIKRRQQKIRKYTMRRLLQETELVEPL
TPSGAMPNQAQMRILKETELRKVKVLGSGAFGTVYKGIWIPDGENVKIPV
AIKVLRENTSPKANKEILDEAYVMAGVGSPPYVSRLLGICLTSTVQLVTQL
MPYGCLLDHVRENRLGSLQDILLNWCMTAKGMSYLEDVRLVHRDLAARN
VLVKSPPNHVKITDFGLARLLDIDETEHADGGKVPIKWMALSIILRRRFT
HQSDVWSYGVTVWELMTFGAKPYDGIPAREIPDLLEKGERLPQPPICTID

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VYIMIMVKCWMIDSECRPRFRELVSFESRMARDPQRFVVIQNEGLGPASPL
DSTFYRSLLEDDDMGDLVDAEEYLVPPQGGFFCPDPAPGAGGMVHHRSS
STRSGGDLTLGLEPSEEEAPRSLAPSEGAGSDVEDGDLGMAAKGLQS
LPTHDPSPQLQRYSEDPTVPLPSETDGYVAPLTCSPQPEYVNPQDVRPQFP
SPREGPLPAARPAAGATLERPKTSLSPGKNGVVKDVFAFGGAVENPEYLTPO
GGAAPQPHPPPAFSPAFDNLYYWDQDPPERGAPPSTFKGTPTAENPEYLG
LDVPV

(18) NCA (CEACAM6, Genbank accession no. M18728); Barnett T., et al. *Genomics* 3, 59-66, 1988; Tawaragi Y., et al. *Biochem. Biophys. Res. Commun.* 150, 89-96, 1988; Strausberg R. L., et al. *Proc. Natl. Acad. Sci. USA.* 99:16899-16903, 2002; WO2004063709; EP1439393 (claim 7); WO2004044178 (Example 4); WO2004031238; WO2003042661 (claim 12); WO200278524 (Example 2); WO200286443 (claim 27; Page 427); WO200260317 (claim 2);

Accession: P40199; Q14920; EMBL; M29541; AAA59915.1. EMBL; M18728;

344 aa (SEQ ID NO: 18)
MGPPSAPPCLRHVPWKEVLLTASLLTFWNPPTAKLTISTPFNVAEKGE
VLLLAHLNLPQNRIGYSWKGERVDGNSLIVGYVIGTQQATPGPAYSGRET
TYPNASLLIQNVNTQNDTGFYTLQVIKSLVNEEATGQFHVYPELKPSPIS
SNNNSNPVEDKDAVFTCEPEVQNTTYLWVWNGQSLPVSRLQLSNGNMTL
TLLSVKRNDAGSYECEIQNPASANRSDPVTNLNVLGPDVPTISPISKANYR
PGENLNLSCHAASNPPAQYSWFINGTFQOSTQELFIPNITVNNSGSYMCO
AHNSATGLNRTTVMITVSGSAPVLSAVATVGITIGVLARVALI

(19) MDP (DPEP1, Genbank accession no. BC017023, *Proc. Natl. Acad. Sci. USA.* 99 (26):16899-16903 (2002)); WO2003016475 (claim 1); WO200264798 (claim 33; Page 85-87); JP05003790 (FIG. 6-8); WO9946284 (FIG. 9);

Cross-references: MIM:179780; AAH17023.1; BC017023_1

411 aa (SEQ ID NO: 19)
MWSGWWLWPLVAVCTADFFRDEAERIMRDSFVIDGHNDLPWQLDMENNR
LQDERANLTLTLAGHTNIPKLRAGFVGGQFWSVYTPCDTQNKDAVVRTLE
QMDVVHRMCRMPETFLYVTSSAGIRQAFREGKVASLIGVEGGHSIDSSL
GVLRLALYQLGMRYLTLTHSCNTPWADNWLVDTDGSEPQSGLSPPGQRRV
KELNRLGVLDLHVSATMKATLQLSRAPVIFSHSSAYSVCASRRNVDP
DVLRLVKQTDLSVMVNFYNNYISCTNKANLSQVADHLHDIKAVAGARAVG
FGGDFDGVPRVPEGLDVSQYPDLIAELLRRNWTEAEVKGALADNLLRVF
EAVEQASNLTAPEEPIPLDQLGGSCTHYGYSSGASSLHRHWGLLLAS
LAPLVLCLSLL

(20) IL20R α (IL20Ra, ZCYTOR7, Genbank accession no. AF184971);

Clark H. F., et al. *Genome Res.* 13, 2265-2270, 2003; Mungall A. J., et al. *Nature* 425, 805-811, 2003; Blumberg

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H., et al. Cell 104, 9-19, 2001; Dumoutier L., et al. J. Immunol. 167, 3545-3549, 2001; Parrish-Novak J., et al. J. Biol. Chem. 277, 47517-47523, 2002; Pletnev S., et al. (2003) Biochemistry 42:12617-12624; Sheikh F., et al. (2004) J. Immunol. 172, 2006-2010; EP1394274 (Example 11); US2004005320 (Example 5); WO2003029262 (Page 74-75); WO2003002717 (claim 2; Page 63); WO200222153 (Page 45-47); US2002042366 (Page 20-21); WO200146261 (Page 57-59); WO200146232 (Page 63-65); WO9837193 (claim 1; Page 55-59); Accession: Q9UHF4; Q6UWA9; Q96SH8; EMBL; AF184971; AAF01320.1.

553 aa

(SEQ ID NO: 20)
MRAPGRPALRPLPLPPLLLLLAAPWGRAVPCVSGGLPKPANITFLSINM
KNVLQWTPPEGLQGVKVTYTVQYFIYGQKKWLNKSECRNINRTYCDLSAE
TSDYEHQYYAKVKAIWGTCKSKWAESGRFYFPLETQIGPPEVALTTDEKS
ISVVLTAPEKWKRNPELPLVSMQQTYSNLKYNVSVLNTKSNRTWSQCVTN
HTLVLTWLEPNTLYCVHVESFVPGPPRAQPSKQCARLTLDKQSEFKAK
IIFWYVLPISITVELFSVMGYSIYRIYHVGKEKHPANLILYIGNEFDKRF
FVPAEKIVINFITLNISSDKISHQDMSLLGKSSDVSSLNDPQPSGNLRP
PQEEEEVKHLGYASHLMEIFCDSEENTEGTSFTQQESLSRTIPPDKTVIE
YEYDVRTTDICAGPEEQELSLQEVSTQGTLLSQAALAVLGPQTLOYSY
TPQLQDLPLAQEHTDSEEGPEEPSTTLVDWDPTGRLCIPSLSSFDQD
SEGCEPSEGDLGEEGLSLRLYEAPADRPGENETYLQMFEWGLYVQ
MEN

(21) Brevican (BCAN, BEHAB, Genbank accession no. AF229053)

Gary S. C., et al. Gene 256, 139-147, 2000; Clark H. F., et al. Genome Res. 13, 2265-2270, 2003; Strausberg R. L., et al. Proc. Natl. Acad. Sci. USA. 99, 16899-16903, 2002; US2003186372 (claim 11); US2003186373 (claim 11); US2003119131 (claim 1; FIG. 52); US2003119122 (claim 1; FIG. 52); US2003119126 (claim 1); US2003119121 (claim 1; FIG. 52); US2003119129 (claim 1); US2003119130 (claim 1); US2003119128 (claim 1; FIG. 52); US2003119125 (claim 1); WO2003016475 (claim 1); WO200202634 (claim 1);

911 aa

(SEQ ID NO: 21)
MAQLFLPLLAALVLAQAPALADVLEGDSSDRAFRVRIAGDAPLQGVLG
GALTIPCHVHYLRPPPSRRAVLGSPRVKWTFLSRGREAEVLVARGVRVKV
NEAYRFRVALPAYPASLTDVLSLSELRPNDSGIYRCEVQHIGIDSSDAV
EVKVGKVVFLYREGSARYAFSFGAQEACARIGAHIAATPEQLYAAVLGGY
EQCDAGWLSQDQTVRYPIQTPREACYGDMGPGVGRNYGVVDPDDLVDVYC
YAEDLNGELFLGDPPEKLTLEEARAYCQERGAEIATTGQLYAAWDGGLDH
CSPGWLADGVSRYPIVTPSQRCGGLPGVKTLFLFPNQTFPNKHSRNFV
YCFRDSAQPSAIPASNPASNPDGLAEIVTVTETLEELQLPQEATESE
SRGAIYSIPIMEDGGGSGSTPEDPAEAPRTLLEFETQSMVPPTGESEEEG
KALEEEEEKYDEEEEEEEEEEEVEDEALWAWPSELSSPGPEASLPTEPA
AQEKSLSQAPARAVLPGASPLPDGESEASRPVRVHGPPTETLPTPRERN

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LASPSPTLVEAREVEGEATGGPELSGVPRGESEETGSSEGAAPSLPATRA
PEGTRELEAPSEDNSGRTPAGTSVQAQVPLPTDSASRGGVAVVPASGDC
VPSPCNHNGTCLLEEEEGVRCLCLPGYGGDLCDVGLRFCNPGWDAFQAGACY
KHFSTRRSWEEAETQCRMYGAHLASISTPEEQDFINNRYREYQWIGLNDR
TIEGDFLWSDGVPLLYENWNPQGPDSYFLSGENCVMVWHDQGWSDVPC
NYHLSYTKMGLVSCGPPPELPLAQVFGPRRLRYEVDTVLRYRCREGLAQ
RNLPLIRCQENGRWEAPQISCVPRPARALHPEDPEGRQLRLGRWKAL
LIPPSSPMMPG

(22) EphB2R (DRT, ERK, Hek5, EPHT3, Tyro5, Genbank accession no. NM_004442) Chan, J. and Watt, V. M., Oncogene 6 (6), 1057-1061 (1991) Oncogene 10 (5):897-905 (1995), *Annu. Rev. Neurosci.* 21:309-345 (1998), *Int. Rev. Cytol.* 196:177-244 (2000); WO2003042661 (claim 12); WO200053216 (claim 1; Page 41); WO2004065576 (claim 1); WO2004020583 (claim 9); WO2003004529 (Page 128-132); WO200053216 (claim 1; Page 42);

Cross-references: MIM:600997; NP_004443.2; NM_004442_1

987 aa

(SEQ ID NO: 22)
MALRRLGAALLLLPLLAEEETLMDSTTATAELGWMVHPSPGWEEVSGYD
ENMNTIRTYQVCNVFESSQNNWLRTKFIIRRGGAHRIHVEMKFSVRDCSSI
PSVPGSCKETFNLYYYEADFDSATKTFPNWMENPWKVDITIADESFSQV
DLGGRVMKINTEVRSGPVSRSGLYAFQDYGGCMLIAVRVEYRKCPRI
IQNGAIFQETLSGAESTSLVAARGSCIANAEEDVPIKLYCNGDGEWLVP
IGRCMCKAGFEAVENGTVCRGCPSTGFKANQGEACTHCIPINSRTTSEGA
TNCVCRNGYYRADLPLDMPCTTIPSAPQAVISSVNETSLMLEWTPPRDS
GGREDLVYNIICKSCSGRGACTRCGDNVQYAPRQLGLETPRIYISDLLA
HTQYTFEIQAVNGVTDQSPFSQFASVNITTNQAAPSASVIMHQVSRVTD
SITLSWSQPDQPNGVILDYELQYYEKELSEYNATAIKSPNTNTVTVQGLKA
GAIYVQVRARTVAGYGRYSGMYFQMTAEYQTSIQEKLPLIIGSSAA
GLVFLIAVVVIAIVCNRRRGFERADSEYTDKQLHYTSGHMTPGMKIYIDP
FTYEDPNEAVREFAKEIDISCVKIEQVIGAGEFGEVCSGHLKLPKREIF
VAIKILKSGYTEKQRDFLSEASIMGQFDHPNVHLEGVVIKSTPVMIIIT
EFMENGSLDSFLRQNDGQFTVIQLVGMRLGIAAGMKYLADMNYVHRDLAA
RNILVNSNLVCKVSDFGLSRFLIEDDISDPTYISALGGKIPIRWTAPEAIQ
YRKFTSASDVWSYGIWMVEVMSYGERPYWMDINQDVINAIEQDYRLPPPM
DCPSALHQLMLDCWQKDRNHRPKFGQIVNILDKMIRNPNSLKAMAPLSSG
INLPLLDRTIPDYTSFNIVDEWLEAIKMGQYKESFANAGFTSFDVVSQMM
MEDILRVGVTLAGHQKILNSIQVMRAQMNQIQSVSEV

(23) ASLG659 (B7h, Genbank accession no. AX092328) US20040101899 (claim 2); WO2003104399 (claim 11); WO2004000221 (FIG. 3); US2003165504 (claim 1); US2003124140 (Example 2); US2003065143 (FIG. 60); WO2002102235 (claim 13; Page 299); US2003091580 (Example 2); WO200210187 (claim 6; FIG. 10); WO200194641 (claim 12; FIG. 7b); WO200202624 (claim 13; FIG. 1A-1B); US2002034749 (claim 54; Page 45-46);

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WO200206317 (Example 2; Page 320-321, claim 34; Page 321-322); WO200271928 (Page 468-469); WO200202587 (Example 1; FIG. 1); WO200140269 (Example 3; Pages 190-192); WO200036107 (Example 2; Page 205-207); WO2004053079 (claim 12); WO2003004989 (claim 1); WO200271928 (Page 233-234, 452-453); WO 0116318;

282 aa (SEQ ID NO: 23)
 MASLGQILFWSTIIIIILAGATAIIIGFGISGRHSITVTTVASAGNIGE
 DGILSCTFEPDIKLSDIVIQWLKEGVLGLVHEFKEGKDELSEQDEMFRGR
 TAVFADQVIVGNASLRLKNVQLTDAGTYKCYIITSKGKKNANLEYKTGAF
 SMPEVNVVDYNASSETLRCEAPRWFPOPTVVWASQVDQGANFSEVSNTSFE
 LNSENVMTKVVSVLVNVTINNTYSCMIENDIAKATGDIKVTSEIKRRSH
 LQLLNSKASLCVSSFFAISWALLPLSPYMLK

(24) PSCA (Prostate stem cell antigen precursor, Genbank accession no. AJ297436) Reiter R. E., et al. Proc. Natl. Acad. Sci. USA. 95, 1735-1740, 1998; Gu Z., et al. Oncogene 19, 1288-1296, 2000; Biochem. Biophys. Res. Commun. (2000) 275(3):783-788; WO2004022709; EP1394274 (Example 11); US2004018553 (claim 17); WO2003008537 (claim 1); WO200281646 (claim 1; Page 164); WO2003003906 (claim 10; Page 288); WO200140309 (Example 1; FIG. 17); US2001055751 (Example 1; FIG. 1b); WO200032752 (claim 18; FIG. 1); WO9851805 (claim 17; Page 97); WO9851824 (claim 10; Page 94); WO9840403 (claim 2; FIG. 1B);

Accession: 043653; EMBL; AF043498; AAC39607.1.

123 aa (SEQ ID NO: 24)
 MKAVLLALLMAGLALPGTALLCYSCKAQVSNEDCLQVENCTQLGEQWCT
 ARIRAVGLLTVISKGCSLNCVDDSDQDYVYGKKNITCCDTLCLNASGAHAL
 QPAAAILALLPALGLLLWGPGQL

(25) GEDA (Genbank accession No. AY260763); AAP14954 lipoma HMGIC fusion-partner-like protein/pid=AAP14954.1—*Homo sapiens* Species: *Homo sapiens* (human)

WO2003054152 (claim 20); WO2003000842 (claim 1); WO2003023013 (Example 3, claim 20); US2003194704 (claim 45);

Cross-references: GI:30102449; AAP14954.1; AY260763_1

236 aa (SEQ ID NO: 25)
 MPGAAAAAAAAAAMLPAGEAAKLYHTNYVRNSPAIGVLWAIPTICFAIV
 NVVCFIQPYWIGDGVDTPOAGYFGLFHYCIGNGFSRELTCRGSFTDFSTL
 PSGAPKAASFFIGLSMMLIIACIICFTLFFFCNTATVYKICAWMQLTSAA
 CLVLGCMIFPDGWDSEVKRMCGEKTDKYTLGACSVRWAYILAIIGILDA
 LILSFLAPVLGNRQDSLMAEELKAENKVLLSQYSLE

(26) BAFF-R (B cell-activating factor receptor, BLYS receptor 3, BR3, Genbank accession No. NP_443177.1); NP_443177 BAFF receptor/pid=NP_443177.1—*Homo sapiens*

Thompson, J. S., et al. Science 293 (5537), 2108-2111 (2001); WO2004058309; WO2004011611; WO2003045422

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(Example; Page 32-33); WO2003014294 (claim 35; FIG. 6B); WO2003035846 (claim 70; Page 615-616); WO200294852 (Col 136-137); WO200238766 (claim 3; Page 133); WO200224909 (Example 3; FIG. 3); Cross-references: MIM:606269; NP_443177.1; NM_052945_1

184 aa (SEQ ID NO: 26)
 MRRGPRSLRGRDAPAPTPCVPAPAECDLLVRHCVACGLLRTPRPKPAGASS
 PAPRTALQPQESVGAGAGEAALPLPGLLFGAPALLGLALVLALVLVGLVS
 WRRRQRRLRGASSAEAPDGDGDAPEPLDKVIIISPGISDATAPAWPPPGGE
 DPGTTPPGHVSVPVATELGSTELVTTKTAGPEQQ

(27) CD22 (B-cell receptor CD22-B isoform, Genbank accession No. NP-001762.1); Stamenkovic, I. and Seed, B., *Nature* 345 (6270), 74-77 (1990); US2003157113; US2003118592; WO2003062401 (claim 9); WO2003072036 (claim 1; FIG. 1); WO200278524 (Example 2); Cross-references: MIM:107266; NP_001762.1; NM_001771_1

847 aa (SEQ ID NO: 27)
 MHLLGPWLLLLVLELAFSDSSKVVFEHPETLYAWEGACVWIPCTYRALD
 GDLESFILFHNPEYNKNTSKFDGTRLTESTKDGKVPSEKQKRVQFLGDKNK
 NCTLSIHPVHLNDSGQLGLRMESKTEKWMERIHLNVSERFPFPHIQLPPE
 IQESQEVTLTCLLNFSCYGYPIQLQWLLEGVPMRQAAVTSTSLTIKSVFT
 RSELKFSPPQWSHHGKIVTCQLQDADGKFLSNDTVQLNVKHTPKLEIKVTP
 SDAIVREGDSVTMTCEVSSSNPEYTTVSWLKDGTSLKKQNTFTLNLREVT
 KDQSGKYCCQVSNVNDVGPGRSEEVFLQVYAPEPSTVQILHSPAVEGSQVE
 FLCMSLANPLNTYTYHNGKEMQGRTEEKVHIPKILPWHAGTYSVCAEN
 ILGTGQRPGAELDVQYPPKVTTVIQNPMPIREGDVTVTLSCNYSSNPS
 VTRYEWKPHGAWEEPGLVGLKIQNVGWDNTTIACARCNSWCWSAPVALN
 VQYAPRDVVRKI KPLSEIHSNGSVSLQCDFFSSSHPEKVFQFWEKNGRLL
 GKESQLNEDSISPEDAGSYSCVWNNSIGQTASKAWTLEVLYAPRRLRVSM
 SPGDQVMEGKSATLTCESDANPPVSHYTWDWNNSLPHHSQKRLRLEPVK
 VQHSWAYWCQGTNSVKGKRSPLSTLTVYYSPEITIGRRVAVGLGSCLAILI
 LAICGLKLQRRWKRTQSQGLQENSSGQSFEVRNKKVRRAPLSEGPBSLG
 CYNPMMEDGISYTTLREPEMNIPRTGDAESSEMQRPRRTCDTDTVYSALH
 KRQVGDYENVIPDFPEDEGIHYSELIQFGVGERPQAQENVYVILKH

(28) CD79a (CD79A, CD79a, immunoglobulin-associated alpha, a B cell-specific protein that covalently interacts with Ig beta (CD79B) and forms a complex on the surface with Ig M molecules, transduces a signal involved in B-cell differentiation) PROTEIN SEQUENCE Full mppgpgv . . . dvqlkqp (1 . . . 226; 226 aa), pI: 4.84, MW: 25028 TM: 2 [P] Gene Chromosome: 19q13.2, Genbank accession No. NP_001774.1;

WO2003088808, US20030228319; WO2003062401 (claim 9); US2002150573 (claim 4, pages 13-14); WO9958658 (claim 13, FIG. 16); WO9207574 (FIG. 1); U.S. Pat. No. 5,644,033; Ha et al. (1992) J. Immunol. 148(5):1526-1531; Mueller et al. (1992) Eur. J. Biochem. 22:1621-1625; Hashimoto et al. (1994) Immunogenetics

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40(4):287-295; Preud'homme et al. (1992) Clin. Exp. Immunol. 90(1):141-146; Yu et al. (1992) J. Immunol. 148(2) 633-637; Sakaguchi et al. (1988) EMBO J. 7(11): 3457-3464;

226 aa (SEQ ID NO: 28)
MPGGPGVLQALPATIFLLFLLSAVYLGPGCQALWMHKVPASLMVSLGEDA
HFQCPHNSNNANIVWRVLHGNYPWPEFLGPGEDPNGTLIIQNVNKS
GGIYVCRVQEGNESYQQSCGYTLVRQPPRPFLDMGEGTKNRIITAE
ILLFCAVVPGTLLFRKRWNQEKLGDLADGEYEDENLYEGLNLDCCSMYE
DISRGLQGTQYQDVGSLNIGDVQLEKP

(29) CXCR5 (Burkitt's lymphoma receptor 1, a G protein-coupled receptor that is activated by the CXCL13 chemokine, functions in lymphocyte migration and humoral defense, plays a role in HIV-2 infection and perhaps development of AIDS, lymphoma, myeloma, and leukemia) PROTEIN SEQUENCE Full mnypltd . . . atsltf (1 . . . 372; 372 aa), pI: 8.54 MW: 41959 TM: 7 [P] Gene Chromosome: 11q23.3, Genbank accession No. NP_001707.1; WO2004040000; WO2004015426; US2003105292 (Example 2); U.S. Pat. No. 6,555,339 (Example 2); WO200261087 (FIG. 1); WO200157188 (claim 20, page 269); WO200172830 (pages 12-13); WO200022129 (Example 1, pages 152-153, Example 2, pages 254-256); WO9928468 (claim 1, page 38); U.S. Pat. No. 5,440,021 (Example 2, col 49-52); WO9428931 (pages 56-58); WO9217497 (claim 7, FIG. 5); Dobner et al. (1992) Eur. J. Immunol. 22:2795-2799; Barella et al. (1995) Biochem. J. 309:773-779;

372 aa (SEQ ID NO: 29)
MNYPLTLEMDLENLEDFWELDRLDNYNDTSLVENHLCPCATEGFLMASFK
AVFVPVAYSILFLGVGNVVLVILERHRQTRSSTETFLFHLAVADLLL
VFILPFAVAEGSWGVLGTFLCKTVIALHKVNFYCSSLLLACIAVDRYLA
IVHAVHAYRHRRLLSIHITCGTIWLVGFLALPEILFAKVSQGHNNSLP
RCTFSQENQAETHAWFTSRFLYHVAGFLPLMLVMGWCVYGVVHRLRQAQR
RPQRQKAVRVAILVTSIFFLCWSPPYHIVIFLDTLARKAVDNTCKLNGSL
PVAITMCEFLGLAHCCNPMLYTFAGVKFRSDLSRLTLKLGCTGPASLCQ
LPFSWRRSSLSSEENATSLTTF

(30) HLA-DOB (Beta subunit of MEW class II molecule (Ia antigen) that binds peptides and presents them to CD4+T lymphocytes) PROTEIN SEQUENCE Full msggwpv . . . vllpqsc (1 . . . 273; 273 aa, pI: 6.56 MW: 30820 TM: 1 [P] Gene Chromosome: 6p21.3, Genbank accession No. NP_002111.1;

Tonnelle et al. (1985) EMBO J. 4(11):2839-2847; Jonsson et al. (1989) Immunogenetics 29(6):411-413; Beck et al. (1992) J. Mol. Biol. 228:433-441; Strausberg et al. (2002) Proc. Natl. Acad. Sci USA 99:16899-16903; Servenius et al. (1987) J. Biol. Chem. 262:8759-8766; Beck et al. (1996) J. Mol. Biol. 255:1-13; Naruse et al. (2002) Tissue Antigens 59:512-519; WO9958658 (claim 13, FIG. 15); U.S. Pat. No. 6,153,408 (Col 35-38); U.S. Pat. No. 5,976,551 (col 168-170); U.S. Pat. No. 6,011,146 (col 145-146); Kasahara et al. (1989) Immunogenetics 30(1):66-68; Larhammar et al. (1985) J. Biol. Chem. 260(26):14111-14119;

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273 aa (SEQ ID NO: 30)
MGSGWVPWVVALLVNLTDLSSMTQGTDSPEDFVIAKADCYFTNGTEKV
5 QFVVRIFIFNLEEYVRFDSDVGMFVALTKLGQPDQAEQWNSRLDRLERSRQA
VDGVCRHNYRLGAPFTVGRKVQPEVTVPERTPLLHQHLLHCSVTGFPY
GDIKIKWFLNGQEERAGVMSTGPIRNGDWTFTQTVVMLEMTEPLGHVYTCL
10 VDHSSLLSPVSVWEWRAQSEYSWRKMLSGIAAFLGLLIFLLVGIQLRAQ
KGYVRTQMSGNEVSRAVLLPQSC

(31) P2X5 (Purinergic receptor P2X ligand-gated ion channel 5, an ion channel gated by extracellular ATP, may be involved in synaptic transmission and neurogenesis, deficiency may contribute to the pathophysiology of idiopathic detrusor instability) PROTEIN SEQUENCE Full mgga-gck . . . lephrst (1 . . . 422; 422 aa), pI: 7.63, MW: 47206 TM: 1 [P] Gene Chromosome: 17p13.3, Genbank accession No. NP_002552.2; Le et al. (1997) FEBS Lett. 418(1-2):195-199; WO2004047749; WO2003072035 (claim 10); Touchman et al. (2000) Genome Res. 10:165-173; WO200222660 (claim 20); WO2003093444 (claim 1); WO2003087768 (claim 1); WO2003029277 (page 82);

422 aa (SEQ ID NO: 31)
MGQAGCKGLCLSLFDYKTEKYVIAKNKKVGLLYRLQLASILAYLVVVVFL
30 IKKGQYQVDVTSLSQSAVITKVKGVAFTNTSDLGQRIWDVADYVIPAQGENV
PFVVTNLIVTPNQRVNCAENEGIPDGACSKSDCHAGEAVTAGNGVKTG
RCLRRENLRARGTCEIFAWCPLETSSRPEEPFLKEADFTIFIKNHIRFPK
35 FNFSKSNVMDVKDRSFLKSCHPGPKNHYPICIFRLGSVIRWAGSDFQDIAL
EGGVIGINIEWNCDLKAASECHPHYSFSLDNKLSKSVSSGYNFRFARY
YRDAAGVEFRTLKAYGIRFDMVNGKGAFFCDLVLIYLIKREFYRDKK
40 YEEVRGLEDSSQEADEASGLGLSEQLTSGPGLGMPEQQLQEPPEAKR
GSSSQKNGSVCPQLLEPHRST

(32) CD72 (B-cell differentiation antigen CD72, Lyb-2) PROTEIN SEQUENCE Full maeaity . . . tafrrpd (1 . . . 359; 359 aa), pI: 8.66, MW: 40225 TM: 1 [P] Gene Chromosome: 9p13.3, Genbank accession No. NP_001773.1; WO2004042346 (claim 65); WO2003026493 (pages 51-52, 57-58); WO200075655 (pages 105-106); Von Hoegen et al. (1990) J. Immunol. 144(12):4870-4877; Strausberg et al. (2002) Proc. Natl. Acad. Sci USA 99:16899-16903;

359 aa (SEQ ID NO: 32)
MAEAITYADLRFFVKAPLKKSISSRLGQDPGADDDGEITYENVQVPAVLGV
55 PSSLASSVLGDKAAVKSEQPTASWRAVTSAPVGRILPCRTTCLRYLLGL
LLTCLLLGVTAICLVRYLQVSSQLQQTNRVLEVTNSSLRQQLRLKITQL
GQSAEDLQGSRRRLAQSQEALQVEQRAHQAAEQQLQACQADRQKTETLQ
SEEQRRALQKLSNMENRLKPFPTCGSADTCCPSGWIMHQKSCFYISLT
SKNQWESQKQCETLSSKLATFSETYPQSHSYFLNSLLPNGSGNSYWTG
LSSNKDWKLTDDTQRTTYAQSSCKNKVHKTWSWWTLESESCRSSLPYIC
EMTAFRFPD

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(33) LY64 (Lymphocyte antigen 64 (RP105), type I membrane protein of the leucine rich repeat (LRR) family, regulates B-cell activation and apoptosis, loss of function is associated with increased disease activity in patients with systemic lupus erythematosus) PROTEIN SEQUENCE Full mafdvc . . . rwyqhi (1 . . . 661; 661 aa), pI: 6.20, MW: 74147 TM: 1 [P] Gene Chromosome: 5q12, Genbank accession No. NP_005573.1;

US2002193567; WO9707198 (claim 11, pages 39-42); Miura et al. (1996) *Genomics* 38(3):299-304; Miura et al. (1998) *Blood* 92:2815-2822; WO2003083047; WO9744452 (claim 8, pages 57-61); WO200012130 (pages 24-26);

661 aa
(SEQ ID NO: 33)
MAPDVSCFFWVFLSAGCKVITSWDQMCIEKANKTYNCENLGLSEIPDT
LPNTTEFLFESFNFLPTIHNRTSRLMNLTFDLTRCQINWIHEDTFQSH
HQLSTLVLTGNPLIFMAETSLNGPKSLKHLFLIQTGISNLEFIPVHNLEN
LESYLGSNHISSIKFPKDFPARNLKVLDFQNNAIHYISREDMRSLEQAI
NLSLNFNGNNVKGIELGAFDSTVFSQSLNFGGTPNLSVIFNGLQNSTQSL
WLGTFEDIDDEDISSAMLKGLCEMSVESLNLQEHFSDISSTTFQCFQTQL
QELDLTATHLKGFLPSGMKGLNLLKVLVSVNHFQDLQCQISAAFPPLSLTHL
YIRGNVKKLHLGVGCKLEKGLNLQTLDSLHNDIEASDCCSLQKLNLSHLQT
LNLSHNEPLGLQSQAFKECPQLELLDLAFTRLHINAPQSPFQNLHLFQVL
NLTYCFDLISNQHLLAGLPVLRHLNLKGNHFQDGTITKINLLQTVGSLEV
LILSSCGLLSIDQQAFHSLGKMSHVDLSHNSLTCDSDLSHLKGIYLN
AANSINIISPRLLPILSQSTINLSHNPLDCTCSNIHFLTWYKENLHKLE
GSEETTANPPSLRGVKLSVDKLSGCGITAIGIFFLIVFLLLAILLFFAV
KYLRLWKYQHI

(34) FCRH1 (Fc receptor-like protein 1, a putative receptor for the immunoglobulin Fc domain that contains C₂ type Ig-like and ITAM domains, may have a role in B-lymphocyte differentiation) PROTEIN SEQUENCE Full mlprll . . . vdyedam (1 . . . 429; 429 aa), pI: 5.28, MW: 46925 TM: 1 [P] Gene Chromosome: 1q21-1q22, Genbank accession No. NP_443170.1; WO2003077836; WO200138490 (claim 6, FIG. 18E-1-18E-2); Davis et al. (2001) *Proc. Natl. Acad. Sci USA* 98(17):9772-9777; WO2003089624 (claim 8); EP1347046 (claim 1); WO2003089624 (claim 7);

429 aa
(SEQ ID NO: 34)
MLPRLLLLICAPLCEPAELFLIASPSHPTGSPVTLTCKMPFLQSSDAQF
QCFPRDTRALGPWSSSPKLQIAAMWKEDTGSYWCQAQTMASKVLRSR
SQINVHRVPVADVSLQTPPGQVMGDRVLICSVAMGTGDTFLWYK
GAVGLNLQSKTQSRSLTAEYIIPSVRESDAEQYVCVAENGYGSPSPGLVS
ITVRIPVSRPILMLRAPRAQAEDVLEHCEALRGSPPILYWFYHEDI
TLGSRAPSGGGASFNLSLTHEHSGNYSCEANGLGAQRSEAVTLNFTV
PTGARSNHLTSGVIEGLSLTLPATVALLFCYGLKRKIGRRSARDPLRS
LPSPLPQEFYTLNSPTPGQLQPIYENVNVVSGDEVYSLAYNQPEQESV
AAETLGTHMEDKVSLDIYSRLRKANITDVEDYEDAM

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(35) IRTA2 (Immunoglobulin superfamily receptor translocation associated 2, a putative immunoreceptor with possible roles in B cell development and lymphomagenesis; deregulation of the gene by translocation occurs in some B cell malignancies) PROTEIN SEQUENCE Full mllw-vil . . . assaphr (1 . . . 977; 977 aa), pI: 6.88 MW: 106468 TM: 1 [P] Gene Chromosome: 1q21, Genbank accession No. NP_112571.1;

WO2003024392 (claim 2, FIG. 97); Nakayama et al. (2000) *Biochem. Biophys. Res. Commun.* 277(1):124-127; WO2003077836; WO200138490 (claim 3, FIG. 18B-1-18B-2);

977 aa
(SEQ ID NO: 35)
MLLVVILLVLPVSGQFARTPRPIIFLQPPWTVFQGERVTLTCKGPREY
SPQKTWYHRYLGKEILRETPDNILEVQESGEYRCQAQGSPLSSPVHLD
20 SSASLILQAPLSVFEQDSVVLRCRAAEVTLNNTIYKNDNLVLAFLNKR
TD FHIPHACLKDNAYRCTGYKESCCPVSSNTVKIQVQEPFTRPVLRASS
FQ PISGNPVTLTCTQLSLERSDVPVLRFRFRDDQTLGLGWSLSPNFQIT
AM 25 WSKDSGFYWCKAATMPHVSIDSPPRSWIQVQIPASHPVLTLSPEKAL
NFE GTKVTLHCETQEDSLRTLRYFYHEGVPLRHKSVRCERGASISFSLT
TENS GNYCYTADNGLGAKPSKAVLSVTVPVSHPVNLSSPEDLIFEGAKV
TLH 30 CEAQRGSLPILYQFHEDAALEERSANSAGGVAISFSLTAHSGNYY
CTA DNGFGPQRSKAVLSITVPVSHPVLTLSAEALTFEGATVTLHCEVQ
RGS PQILYQFYHEDMPLWSSSTPSVGRVSFSFSLTEHSGNYYCTADN
GFGPQ 35 RSEVVSLEFVTVPSRPILTLRVPRQAQVVGDLLEHCEAPRGS
PPILYWF YHEDVTLGSSSAPSGGEASFNLSLTAHSGNYSCEANGLVAQ
HSDTISL SVIVPVSRLTPRAPRAQAVVGDLLEHCEALRGSSPILYWFYH
EDVTL GKISAPSGGGASFNLSLTHEHSGIYSCADNGPEAQRSEMTLV
KVAVPVS 40 RPVLTLRAPGTHAAVGDLEHCEALRGSPILYRFFHEDVTLGNR
SSPS GGASLNLSTAEHSGNYSCEADNGLGAQRSETVTLTYITGLTANR
SGPFAT GVAGGLLSIAGLAAGALLLYCWLRSKAGRKASDPARSPDSDS
QEPTYH 45 NVPAWEELQPVYTANPRGENVVYSEVRITQEKKKHAVASDPR
HLRNKGS PIYSEVKVASTPVSGSLFLASSAPHR

See also: WO04/045516 (3 Jun. 2004); WO03/000113 (3 Jan. 2003); WO02/016429 (28 Feb. 2002); WO02/16581 (28 Feb. 2002); WO03/024392 (27 Mar. 2003); WO04/016225 (26 Feb. 2004); WO01/40309 (7 Jun. 2001), and U.S. Provisional patent application Ser. No. 60/520,842 "COMPOSITIONS AND METHODS FOR THE TREATMENT OF TUMOR OF HEMATOPOIETIC ORIGIN", filed 17 Nov. 2003; all of which are incorporated herein by reference in their entirety.

In an embodiment, the Ligand-Linker-Drug Conjugate has Formula IIIa, where the Ligand is an antibody Ab including one that binds at least one of CD30, CD40, CD70, Lewis Y antigen, w=0, y=0, and D has Formula Ib. Exemplary Conjugates of Formula IIIa include where R¹⁷ is $-(CH_2)_5-$. Also included are such Conjugates of Formula IIIa in which D has the structure of Compound 2 in Example 3 and esters thereof. Also included are such Conjugates of Formula IIIa containing about 3 to about 8, in one aspect, about 3 to about 5 Drug moieties D, that is, Conjugates of

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Formula Ia wherein p is a value in the range about 3-8, for example about 3-5. Conjugates containing combinations of the structural features noted in this paragraph are also contemplated as within the scope of the compounds of the invention.

In another embodiment, the Ligand-Linker-Drug Conjugate has Formula IIIa, where Ligand is an Antibody Ab that binds one of CD30, CD40, CD70, Lewis Y antigen, w=1, y=0, and D has Formula Ib. Included are such Conjugates of Formula IIIa in which W is $-(CH_2)_5-$. Also included are such Conjugates of Formula IIIa in which W is -Val-Cit-, and/or where D has the structure of Compound 2 in Example 3 and esters thereof. Also included are such Conjugates of Formula IIIa containing about 3 to about 8, preferably about 3 to about 5 Drug moieties D, that is, Conjugates of Formula Ia wherein p is a value in the range of about 3-8, preferably about 3-5. Conjugates containing combinations of the structural features noted in this paragraph are also exemplary.

In an embodiment, the Ligand-Linker-Drug Conjugate has Formula IIIa, where the Ligand is an Antibody Ab that binds one of CD30, CD40, CD70, Lewis Y antigen, w=1, y=1, and D has Formula Ib. Included are Conjugates of Formula IIIa in which R¹⁷ is $-(CH_2)_5-$. Also included are such Conjugates of Formula IIIa where: W is -Val-Cit-; Y has Formula X; D has the structure of Compound 2 in Example 3 and esters thereof; p is about 3 to about 8, preferably about 3 to about 5 Drug moieties D. Conjugates containing combinations of the structural features noted in this paragraph are also contemplated within the scope of the compounds of the invention.

A further embodiment is an antibody drug conjugate (ADC), or a pharmaceutically acceptable salt or solvate thereof, wherein Ab is an antibody that binds one of the tumor-associated antigens (1)-(35) noted above (the "TAA Compound").

Another embodiment is the TAA Compound or pharmaceutically acceptable salt or solvate thereof that is in isolated and purified form.

Another embodiment is a method for killing or inhibiting the multiplication of a tumor cell or cancer cell comprising administering to a patient, for example a human with a hyperproliferative disorder, an amount of the TAA Compound or a pharmaceutically acceptable salt or solvate thereof, said amount being effective to kill or inhibit the multiplication of a tumor cell or cancer cell.

Another embodiment is a method for treating cancer comprising administering to a patient, for example a human with a hyperproliferative disorder, an amount of the TAA Compound or a pharmaceutically acceptable salt or solvate thereof, said amount being effective to treat cancer, alone or together with an effective amount of an additional anticancer agent.

Another embodiment is a method for treating an autoimmune disease, comprising administering to a patient, for example a human with a hyperproliferative disorder, an amount of the TAA Compound or a pharmaceutically acceptable salt or solvate thereof, said amount being effective to treat an autoimmune disease.

The antibodies suitable for use in the invention can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or by recombinant expression, and are preferably produced by recombinant expression techniques.

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9.5.1 Production of Recombinant Antibodies

Antibodies of the invention can be produced using any method known in the art to be useful for the synthesis of antibodies, in particular, by chemical synthesis or by recombinant expression.

Recombinant expression of antibodies, or fragment, derivative or analog thereof, requires construction of a nucleic acid that encodes the antibody. If the nucleotide sequence of the antibody is known, a nucleic acid encoding the antibody may be assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier et al., 1994, *BioTechniques* 17:242), which involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligation of those oligonucleotides, and then amplification of the ligated oligonucleotides, e.g., by PCR.

Alternatively, a nucleic acid molecule encoding an antibody can be generated from a suitable source. If a clone containing the nucleic acid encoding the particular antibody is not available, but the sequence of the antibody is known, a nucleic acid encoding the antibody can be obtained from a suitable source (e.g., an antibody cDNA library, or cDNA library generated from any tissue or cells expressing the immunoglobulin) by, e.g., PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence.

If an antibody that specifically recognizes a particular antigen is not commercially available (or a source for a cDNA library for cloning a nucleic acid encoding such an immunoglobulin), antibodies specific for a particular antigen can be generated by any method known in the art, for example, by immunizing a patient, or suitable animal model such as a rabbit or mouse, to generate polyclonal antibodies or, more preferably, by generating monoclonal antibodies, e.g., as described by Kohler and Milstein (1975, *Nature* 256:495-497) or, as described by Kozbor et al. (1983, *Immunology Today* 4:72) or Cole et al. (1985 in *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96). Alternatively, a clone encoding at least the Fab portion of the antibody can be obtained by screening Fab expression libraries (e.g., as described in Huse et al., 1989, *Science* 246:1275-1281) for clones of Fab fragments that bind the specific antigen or by screening antibody libraries (See, e.g., Clackson et al., 1991, *Nature* 352:624; Hane et al., 1997 *Proc. Natl. Acad. Sci. USA* 94:4937).

Once a nucleic acid sequence encoding at least the variable domain of the antibody is obtained, it can be introduced into a vector containing the nucleotide sequence encoding the constant regions of the antibody (see, e.g., International Publication No. WO 86/05807; WO 89/01036; and U.S. Pat. No. 5,122,464). Vectors containing the complete light or heavy chain that allow for the expression of a complete antibody molecule are available. Then, the nucleic acid encoding the antibody can be used to introduce the nucleotide substitutions or deletion necessary to substitute (or delete) the one or more variable region cysteine residues participating in an intrachain disulfide bond with an amino acid residue that does not contain a sulphydryl group. Such modifications can be carried out by any method known in the art for the introduction of specific mutations or deletions in a nucleotide sequence, for example, but not limited to, chemical mutagenesis and in vitro site directed mutagenesis (Hutchinson et al., 1978, 1 *Biol. Chem.* 253:6551).

In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., 1984, *Proc. Natl. Acad. Sci.* 81:851-855; Neuberger et al., 1984, *Nature* 312:

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604-608; Takeda et al., 1985, *Nature* 314:452-454) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. A chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region, e.g., humanized antibodies.

Alternatively, techniques described for the production of single chain antibodies (U.S. Pat. No. 4,694,778; Bird, 1988, *Science* 242:423-42; Huston et al., 1988, *Proc. Natl. Acad. Sci. USA* 85:5879-5883; and Ward et al., 1989, *Nature* 334:544-54) can be adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in *E. coli* may also be used (Skerra et al., 1988, *Science* 242:1038-1041).

Antibody fragments that recognize specific epitopes can be generated by known techniques. For example, such fragments include, but are not limited to the F(ab')₂ fragments that can be produced by pepsin digestion of the antibody molecule and the Fab fragments that can be generated by reducing the disulfide bridges of the F(ab')₂ fragments.

Once a nucleic acid sequence encoding an antibody has been obtained, the vector for the production of the antibody can be produced by recombinant DNA technology using techniques well known in the art. Methods that are well known to those skilled in the art can be used to construct expression vectors containing the antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. See, for example, the techniques described in Sambrook et al. (1990, *Molecular Cloning, A Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.) and Ausubel et al. (eds., 1998, *Current Protocols in Molecular Biology*, John Wiley & Sons, NY).

An expression vector comprising the nucleotide sequence of an antibody or the nucleotide sequence of an antibody can be transferred to a host cell by conventional techniques (e.g., electroporation, liposomal transfection, and calcium phosphate precipitation), and the transfected cells are then cultured by conventional techniques to produce the antibody. In specific embodiments, the expression of the antibody is regulated by a constitutive, an inducible or a tissue, specific promoter.

The host cells used to express the recombinant antibody can be either bacterial cells such as *Escherichia coli*, or, preferably, eukaryotic cells, especially for the expression of whole recombinant immunoglobulin molecule. In particular, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for immunoglobulins (Foecking et al., 1988, *Gene* 45:101; Cockett et al., 1990, *BioTechnology* 8:2). A variety of host-expression vector systems can be utilized to express the immunoglobulin antibodies. Such host-expression systems represent vehicles by which the coding sequences of the antibody can be produced and subsequently purified, but also represent cells that can, when transformed or transfected with the appropriate nucleotide coding sequences, express an anti-

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body immunoglobulin molecule in situ. These include, but are not limited to, microorganisms such as bacteria (e.g., *E. coli* and *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing immunoglobulin coding sequences; yeast (e.g., *Saccharomyces Pichia*) transformed with recombinant yeast expression vectors containing immunoglobulin coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing the immunoglobulin coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus (CaMV) and tobacco mosaic virus (TMV)) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing immunoglobulin coding sequences; or mammalian cell systems (e.g., COS, CHO, BH, 293, 293T, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter).

In bacterial systems, a number of expression vectors can be advantageously selected depending upon the use intended for the antibody being expressed. For example, when a large quantity of such a protein is to be produced, vectors that direct the expression of high levels of fusion protein products that are readily purified might be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther et al., 1983, *EMBO J.* 2:1791), in which the antibody coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, 1985, *Nucleic Acids Res.* 13:3101-3109; Van Heeke & Schuster, 1989, *J Biol. Chem.* 264:5503-5509); and the like. pGEX Vectors can also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to a matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) or the analogous virus from *Drosophila melanogaster* is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody coding sequence can be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

In mammalian host cells, a number of viral-based expression systems can be utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest can be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene can then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) results in a recombinant virus that is viable and capable of expressing the immunoglobulin molecule in infected hosts. (e.g., see Logan & Shenk, 1984, *Proc. Natl. Acad. Sci. USA* 81:355-359). Specific initiation signals can also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence

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to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression can be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., 1987, *Methods in Enzymol.* 153:51-544).

In addition, a host cell strain can be chosen to modulate the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products can be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells that possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product can be used. Such mammalian host cells include, but are not limited to, CHO, VERY, BH, HeLa, COS, MDCK, 293, 293T, 3T3, W138, BT483, Hs578T, HTB2, BT20 and T47D, CRL7030 and Hs578Bst.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines that stably express an antibody can be engineered. Rather than using expression vectors that contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells can be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci that in turn can be cloned and expanded into cell lines. This method can advantageously be used to engineer cell lines which express the antibody. Such engineered cell lines can be particularly useful in screening and evaluation of tumor antigens that interact directly or indirectly with the antibody.

A number of selection systems can be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., 1977, *Cell* 11:223), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, 192, *Proc. Natl. Acad. Sci. USA* 48:202), and adenine phosphoribosyltransferase (Lowy et al., 1980, *Cell* 22:817) genes can be employed in tk-, hgp^rt- or ap^rt-cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: DHFR, which confers resistance to methotrexate (Wigler et al., 1980, *Proc. Natl. Acad. Sci. USA* 77:357; O'Hare et al., 1981, *Proc. Natl. Acad. Sci. USA* 78:1527); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, 1981, *Proc. Natl. Acad. Sci. USA* 78:2072); neo, which confers resistance to the aminoglycoside G-418 (*Clinical Pharmacology* 12:488-505; Wu and Wu, 1991, *Biotherapy* 3:87-95; Tolstoshev, 1993, *Ann. Rev. Pharmacol. Toxicol.* 32:573-596; Mulligan, 1993, *Science* 260:926-932; and Morgan and Anderson, 1993, *Ann. Rev. Biochem.* 62:191-217; May, 1993, *TIB TECH* 11(5):155-215) and hyg^r, which confers resistance to hygromycin (Santerre et al., 1984, *Gene* 30:147). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds., 1993, *Current Protocols in Molecular Biology*, John Wiley

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& Sons, NY; Kriegler, 1990, *Gene Transfer and Expression, A Laboratory Manual*, Stockton Press, NY; and in Chapters 12 and 13, Dracopoli et al. (eds), 1994, *Current Protocols in Human Genetics*, John Wiley & Sons, NY.; Colberre-Garapin et al., 1981, *J. Mol. Biol.* 150:1).

The expression levels of an antibody can be increased by vector amplification (for a review, see Bebbington and Hentschel, *The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning*, Vol. 3. (Academic Press, New York, 1987)). When a marker in the vector system expressing an antibody is amplifiable, an increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the nucleotide sequence of the antibody, production of the antibody will also increase (Crouse et al., 1983, *Mol. Cell. Biol.* 3:257).

The host cell can be co-transfected with two expression vectors, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors can contain identical selectable markers that enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector can be used to encode both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, 1986, *Nature* 322:52; Kohler, 1980, *Proc. Natl. Acad. Sci. USA* 77:2197). The coding sequences for the heavy and light chains can comprise cDNA or genomic DNA.

Once the antibody has been recombinantly expressed, it can be purified using any method known in the art for purification of an antibody, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins.

In yet another exemplary embodiment, the antibody is a monoclonal antibody.

In any case, the hybrid antibodies have a dual specificity, preferably with one or more binding sites specific for the hapten of choice or one or more binding sites specific for a target antigen, for example, an antigen associated with a tumor, an autoimmune disease, an infectious organism, or other disease state.

9.5.2 Production of Antibodies

The production of antibodies will be illustrated with reference to anti-CD30 antibodies but it will be apparent for those skilled in the art that antibodies to other members of the TNF receptor family can be produced and modified in a similar manner. The use of CD30 for the production of antibodies is exemplary only and not intended to be limiting.

The CD30 antigen to be used for production of antibodies may be, e.g., a soluble form of the extracellular domain of CD30 or a portion thereof, containing the desired epitope. Alternatively, cells expressing CD30 at their cell surface (e.g., L540 (Hodgkin's lymphoma derived cell line with a T cell phenotype) and L428 (Hodgkin's lymphoma derived cell line with a B cell phenotype)) can be used to generate antibodies. Other forms of CD30 useful for generating antibodies will be apparent to those skilled in the art.

In another exemplary embodiment, the ErbB2 antigen to be used for production of antibodies may be, e.g., a soluble form of the extracellular domain of ErbB2 or a portion thereof, containing the desired epitope. Alternatively, cells expressing ErbB2 at their cell surface (e.g., NIH-3T3 cells transformed to overexpress ErbB2; or a carcinoma cell line

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such as SK-BR-3 cells, see Stancovski et al. *Proc. Natl. Acad. Sci. USA* 88:8691-8695 (1991)) can be used to generate antibodies. Other forms of ErbB2 useful for generating antibodies will be apparent to those skilled in the art.

(i) Polyclonal Antibodies

Polyclonal antibodies are preferably raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the relevant antigen and an adjuvant. It may be useful to conjugate the relevant antigen to a protein that is immunogenic in the species to be immunized, e.g., keyhole limpet hemocyanin, serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor using a bifunctional or derivatizing agent, for example, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxy-succinimide (through lysine residues), glutaraldehyde, succinic anhydride, SOCl_2 , or $\text{R}^1\text{N}=\text{C}=\text{NR}$, where R and R^1 are different alkyl groups.

Animals are immunized against the antigen, immunogenic conjugates, or derivatives by combining, e.g., 100 μg or 5 μg of the protein or conjugate (for rabbits or mice, respectively) with 3 volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later the animals are boosted with $\frac{1}{5}$ to $\frac{1}{10}$ the original amount of peptide or conjugate in Freund's complete adjuvant by subcutaneous injection at multiple sites. Seven to 14 days later the animals are bled and the serum is assayed for antibody titer. Animals are boosted until the titer plateaus. Preferably, the animal is boosted with the conjugate of the same antigen, but conjugated to a different protein and/or through a different cross-linking reagent. Conjugates also can be made in recombinant cell culture as protein fusions. Also, aggregating agents such as alum are suitably used to enhance the immune response.

(ii) Monoclonal Antibodies

Monoclonal antibodies are obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts. Thus, the modifier "monoclonal" indicates the character of the antibody as not being a mixture of discrete antibodies.

For example, the monoclonal antibodies may be made using the hybridoma method first described by Kohler et al., *Nature*, 256:495 (1975), or may be made by recombinant DNA methods (U.S. Pat. No. 4,816,567).

In the hybridoma method, a mouse or other appropriate host animal, such as a hamster, is immunized as hereinabove described to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the protein used for immunization. Alternatively, lymphocytes may be immunized in vitro. Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, *Monoclonal Antibodies: Principles and Practice*, pp. 59-103 (Academic Press, 1986)).

The hybridoma cells thus prepared are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

Preferred myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a

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medium such as HAT medium. Among these, preferred myeloma cell lines are murine myeloma lines, such as those derived from MOPC-21 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, Calif. USA, and SP-2 or X63-Ag8-653 cells available from the American Type Culture Collection, Rockville, Md. USA. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, *J. Immunol.*, 133:3001 (1984); and Brodeur et al., *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)).

Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an in vitro binding assay, such as radioimmunoassay (MA) or enzyme-linked immunosorbent assay (ELISA). The binding affinity of the monoclonal antibody can, for example, be determined by the Scatchard analysis of Munson et al., *Anal. Biochem.*, 107: 220 (1980).

After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, *Monoclonal Antibodies: Principles and Practice*, pp. 59-103 (Academic Press, 1986)). Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown in vivo as ascites tumors in an animal.

The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional antibody purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host cells such as *E. coli* cells, simian COS cells, Chinese Hamster Ovary (CHO) cells, or myeloma cells that do not otherwise produce antibody protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Review articles on recombinant expression in bacteria of DNA encoding the antibody include Skerra et al., *Curr. Opinion in Immunol.*, 5:256-262 (1993) and Plückthun, *Immunol. Revs.*, 130:151-188 (1992).

In a further embodiment, monoclonal antibodies or antibody fragments can be isolated from antibody phage libraries generated using the techniques described in McCafferty et al., *Nature*, 348:552-554 (1990). Clackson et al., *Nature*, 352:624-628 (1991) and Marks et al., *J. Mol. Biol.*, 222: 581-597 (1991) describe the isolation of murine and human antibodies, respectively, using phage libraries. Subsequent publications describe the production of high affinity (nM range) human antibodies by chain shuffling (Marks et al., *Bio/Technology*, 10:779-783 (1992)), as well as combinatorial infection and in vivo recombination as a strategy for constructing very large phage libraries (Waterhouse et al., *Nuc. Acids. Res.*, 21:2265-2266 (1993)). Thus, these tech-

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niques are viable alternatives to traditional monoclonal antibody hybridoma techniques for isolation of monoclonal antibodies.

The DNA also may be modified, for example, by substituting the coding sequence for human heavy chain and light chain constant domains in place of the homologous murine sequences (U.S. Pat. No. 4,816,567; and Morrison, et al. (1984) *Proc. Natl Acad. Sci. USA* 81:6851), or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide.

Typically such non-immunoglobulin polypeptides are substituted for the constant domains of an antibody, or they are substituted for the variable domains of one antigen-combining site of an antibody to create a chimeric bivalent antibody comprising one antigen-combining site having specificity for an antigen and another antigen-combining site having specificity for a different antigen.

(iii) Humanized Antibodies

A humanized antibody may have one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones et al., *Nature* 321:522-525 (1986); Riechmann et al., *Nature*, 332:323-327 (1988); Verhoeven et al., *Science* 239:1534-1536 (1988)), by substituting hypervariable region sequences for the corresponding sequences of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (U.S. Pat. No. 4,816,567) wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some hypervariable region residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is very important to reduce antigenicity. According to the so-called "best-fit" method, the sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable-domain sequences. The human sequence which is closest to that of the rodent is then accepted as the human framework region (FR) for the humanized antibody (Sims et al., *J. Immunol.*, 151:2296 (1993); Chothia et al., *J. Mol. Biol.*, 196:901 (1987)). Another method uses a particular framework region derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies (Carter et al., *Proc. Natl. Acad. Sci. USA*, 89:4285 (1992); Presta et al., *J. Immunol.*, 151:2623 (1993)).

In another embodiment, the antibodies may be humanized with retention of high affinity for the antigen and other favorable biological properties. Humanized antibodies may be prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to

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bind its antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the hypervariable region residues are directly and most substantially involved in influencing antigen binding.

Various forms of the humanized antibody are contemplated. For example, the humanized antibody may be an antibody fragment, such as a Fab. Alternatively, the humanized antibody may be an intact antibody, such as an intact IgG1 antibody.

The Examples describe production of an exemplary humanized anti-ErbB2 antibody. The humanized antibody may, for example, comprise nonhuman hypervariable region residues incorporated into a human variable heavy domain and may further comprise a framework region (FR) substitution at a position selected from the group consisting of 69H, 71H and 73H utilizing the variable domain numbering system set forth in Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991). In one embodiment, the humanized antibody comprises FR substitutions at two or all of positions 69H, 71H and 73H. Another Example describes preparation of purified trastuzumab antibody from the HERCEPTIN® formulation.

(iv) Human Antibodies

As an alternative to humanization, human antibodies can be generated. For example, it is now possible to produce transgenic animals (e.g., mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (JO gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, e.g., Jakobovits et al., *Proc. Natl. Acad. Sci. USA*, 90:2551 (1993); Jakobovits et al., *Nature*, 362:255-258 (1993); Bruggermann et al., *Year in Immunol.*, 7:33 (1993); and U.S. Pat. Nos. 5,591,669, 5,589,369 and 5,545,807.

Alternatively, phage display technology (McCafferty et al., *Nature* 348:552-553 (1990)) can be used to produce human antibodies and antibody fragments in vitro, from immunoglobulin variable (V) domain gene repertoires from unimmunized donors. According to this technique, antibody V domain genes are cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. Thus, the phage mimics some of the properties of the B-cell. Phage display can be performed in a variety of formats; for their review see, e.g., Johnson, Kevin S. and Chiswell, David J., *Current Opinion in Structural Biology* 3:564-571 (1993). Several sources of V-gene segments can be used for phage display. Clackson et al., *Nature*, 352:624-628 (1991) isolated a diverse array of anti-oxazolone antibodies from a small random combinatorial library of V genes derived from the spleens of immunized mice. A repertoire of V genes from unimmunized human donors can be constructed and antibodies to a diverse array of antigens (including self-antigens) can be isolated essentially following the techniques

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described by Marks et al., *J. Mol. Biol.* 222:581-597 (1991), or Griffith et al., *EMBO J.* 12:725-734 (1993). See, also, U.S. Pat. Nos. 5,565,332 and 5,573,905. As discussed above, human antibodies may also be generated by in vitro activated B cells (see U.S. Pat. Nos. 5,567,610 and 5,229,275). Human anti-CD30 antibodies are described in U.S. patent application Ser. No. 10/338,366.

(v) Antibody Fragments

Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, e.g., Morimoto et al., *Journal of Biochemical and Biophysical Methods* 24:107-117 (1992); and Brennan et al., *Science*, 229:81 (1985)). However, these fragments can now be produced directly by recombinant host cells. For example, the antibody fragments can be isolated from the antibody phage libraries discussed above. Alternatively, Fab'-SH fragments can be directly recovered from *E. coli* and chemically coupled to form F(ab')₂ fragments (Carter et al., *Bio/Technology* 10:163-167 (1992)). According to another approach, F(ab')₂ fragments can be isolated directly from recombinant host cell culture. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner. In other embodiments, the antibody of choice is a single chain Fv fragment (scFv). See WO 93/16185; U.S. Pat. Nos. 5,571,894; and 5,587,458. The antibody fragment may also be a "linear antibody", e.g., as described in U.S. Pat. No. 5,641,870 for example. Such linear antibody fragments may be monospecific or bispecific.

(vi) Bispecific Antibodies

Bispecific antibodies are antibodies that have binding specificities for at least two different epitopes. Exemplary bispecific antibodies may bind to two different epitopes of the CD30 protein. Alternatively, an anti-CD30 arm may be combined with an arm which binds to a Fc receptors for IgG (FcγR), such as FcγRI (CD64), FcγRII (CD32) and FcγRIII (CD16) so as to focus cellular defense mechanisms to the CD30-expressing cell. Bispecific antibodies may also be used to localize cytotoxic agents to cells which express CD30.

Traditional production of full length bispecific antibodies is based on the coexpression of two immunoglobulin heavy chain-light chain pairs, where the two chains have different specificities (Millstein et al., *Nature*, 305:537-539 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. Purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829, and in Traunecker et al., *EMBO J.* 10:3655-3659 (1991). According to a different approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant domain sequences. The fusion preferably is with an immunoglobulin heavy chain constant domain, comprising at least part of the hinge, CH₂, and CH₃ regions. It is preferred to have the first heavy-chain constant region (CH₁) containing the site necessary for light chain binding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide

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fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance.

In one embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. This approach is disclosed in WO 94/04690. For further details of generating bispecific antibodies see, for example, Suresh et al., *Methods in Enzymology*, 121:210 (1986).

According to another approach described in U.S. Pat. No. 5,731,168, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant cell culture. The preferred interface comprises at least a part of the CH₃ domain of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (e.g., tyrosine or tryptophan). Compensatory "cavities" of identical or similar size to the large side chain(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (e.g., alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

Techniques for generating bispecific antibodies from antibody fragments have also been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. Brennan et al., *Science*, 229: 81 (1985) describe a procedure wherein intact antibodies are proteolytically cleaved to generate F(ab')₂ fragments. These fragments are reduced in the presence of the dithiol complexing agent sodium arsenite to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-thiol by reduction with mercaptoethylamine and is mixed with an equimolar amount of the other Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

Recent progress has facilitated the direct recovery of Fab'-SH fragments from *E. coli*, which can be chemically coupled to form bispecific antibodies. Shalaby et al., *J. Exp. Med.*, 175: 217-225 (1992) describe the production of a fully humanized bispecific antibody F(ab')₂ molecule. Each Fab' fragment was separately secreted from *E. coli* and subjected to directed chemical coupling in vitro to form the bispecific antibody.

Various techniques for making and isolating bispecific antibody fragments directly from recombinant cell culture have also been described. For example, bispecific antibodies have been produced using leucine zippers. Kostelny et al., *J. Immunol.*, 148(5):1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to

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form monomers and then re-oxidized to form the antibody heterodimers. This method can also be utilized for the production of antibody homodimers. The "diabody" technology described by Hollinger et al., *Proc. Natl. Acad. Sci. USA*, 90:6444-6448 (1993) has provided an alternative mechanism for making bispecific antibody fragments. The fragments comprise a heavy-chain variable domain (VH) connected to a light-chain variable domain (VL) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the VH and VL domains of one fragment are forced to pair with the complementary VL and VH domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See Gruber et al., *J. Immunol.*, 152:5368 (1994).

Antibodies with more than two valencies are contemplated. For example, trispecific antibodies can be prepared. Tutt et al. *J. Immunol.* 147: 60 (1991).

(vii) Other Amino Acid Sequence Modifications

Amino acid sequence modification(s) of the antibodies described herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of the antibodies are prepared by introducing appropriate nucleotide changes into the antibody nucleic acid, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of, residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution is made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid changes also may alter post-translational processes of the antibody, such as changing the number or position of glycosylation sites.

A useful method for identification of certain residues or regions of the antibody that are favored locations for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells *Science*, 244:1081-1085 (1989). Here, a residue or group of target residues are identified (e.g., charged residues such as arg, asp, his, lys, and glu) and replaced by a neutral or negatively charged amino acid (most preferably alanine or polyalanine) to affect the interaction of the amino acids with antigen. Those amino acid locations demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants at, or for, the sites of substitution. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation per se need not be predetermined. For example, to analyze the performance of a mutation at a given site, ala scanning or random mutagenesis is conducted at the target codon or region and the expressed antibody variants are screened for the desired activity.

Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue or the antibody fused to a cytotoxic polypeptide. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme (e.g., for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

Another type of variant is an amino acid substitution variant. These variants have at least one amino acid residue

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in the antibody molecule replaced by a different residue. The sites of greatest interest for substitutional mutagenesis include the hypervariable regions, but FR alterations are also contemplated.

Substantial modifications in the biological properties of the antibody are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally-occurring residues are divided into groups based on common side-chain properties:

- (1) hydrophobic: norleucine, met, ala, val, leu, ile;
- (2) neutral hydrophilic: cys, ser, thr;
- (3) acidic: asp, glu;
- (4) basic: asn, gln, his, lys, arg;
- (5) residues that influence chain orientation: gly, pro; and
- (6) aromatic: trp, tyr, phe.

Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

A particularly preferred type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (e.g., a humanized or human antibody). Generally, the resulting variant(s) selected for further development will have improved biological properties relative to the parent antibody from which they are generated. A convenient way for generating such substitutional variants involves affinity maturation using phage display. Briefly, several hypervariable region sites (e.g., 6-7 sites) are mutated to generate all possible amino substitutions at each site. The antibody variants thus generated are displayed in a monovalent fashion from filamentous phage particles as fusions to the gene III product of M13 packaged within each particle. The phage-displayed variants are then screened for their biological activity (e.g., binding affinity) as herein disclosed. In order to identify candidate hypervariable region sites for modification, alanine scanning mutagenesis can be performed to identify hypervariable region residues contributing significantly to antigen binding. Alternatively, or additionally, it may be beneficial to analyze a crystal structure of the antigen-antibody complex to identify contact points between the antibody and the antigen. Such contact residues and neighboring residues are candidates for substitution according to the techniques elaborated herein. Once such variants are generated, the panel of variants is subjected to screening as described herein and antibodies with superior properties in one or more relevant assays may be selected for further development.

It may be desirable to modify the antibody of the invention with respect to effector function, e.g., so as to enhance antigen-dependent cell-mediated cytotoxicity (ADCC) and/or complement dependent cytotoxicity (CDC) of the antibody. This may be achieved by introducing one or more amino acid substitutions in an Fc region of the antibody. Alternatively or additionally, cysteine residue(s) may be introduced in the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus generated may have improved internalization capability and/or increased complement-mediated cell killing and antibody-dependent cellular cytotoxicity (ADCC). See Caron et al. *J. Exp. Med.* 176:1191-1195 (1992) and Shopes, B. J. *Immunol.* 148:2918-2922 (1992). Homodimeric antibodies with enhanced anti-tumor activity may also be prepared using heterobifunctional cross-linkers as described in Wolff et al. *Cancer Research* 53:2560-2565 (1993). Alternatively, an antibody can be engineered which

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has dual Fc regions and may thereby have enhanced complement lysis and ADCC capabilities. See Stevenson et al. *Anti-Cancer Drug Design* 3:219-230 (1989).

To increase the serum half life of the antibody, one may incorporate a salvage receptor binding epitope into the antibody (especially an antibody fragment) as described in U.S. Pat. No. 5,739,277, for example. As used herein, the term "salvage receptor binding epitope" refers to an epitope of the Fc region of an IgG molecule (e.g., IgG₁, IgG₂, IgG₃, or IgG₄) that is responsible for increasing the in vivo serum half-life of the IgG molecule.

(viii) Glycosylation Variants

Antibodies in the ADC of the invention may be glycosylated at conserved positions in their constant regions (Jef- 5 feris and Lund, (1997) *Chem. Immunol.* 65:111-128; Wright and Morrison, (1997) *TibTECH* 15:26-32). The oligosaccharide side chains of the immunoglobulins affect the protein's function (Boyd et al., (1996) *Mol. Immunol.* 32:1311-1318; Wittwe and Howard, (1990) *Biochem.* 29:4175-4180), and the intramolecular interaction between portions of the glycoprotein which can affect the conformation and presented three-dimensional surface of the glycoprotein (Hef- 10 feris and Lund, supra; Wyss and Wagner, (1996) *Current Opin. Biotech.* 7:409-416). Oligosaccharides may also serve to target a given glycoprotein to certain molecules based upon specific recognition structures. For example, it has been reported that in agalactosylated IgG, the oligosaccharide moiety 'flips' out of the inter-CH₂ space and terminal N-acetylglucosamine residues become available to bind mannose binding protein (Malhotra et al., (1995) *Nature Med.* 1:237-243). Removal by glycopeptidase of the oligosaccharides from CAMPATH-1H (a recombinant humanized murine monoclonal IgG1 antibody which recognizes the CDw52 antigen of human lymphocytes) produced in Chinese Hamster Ovary (CHO) cells resulted in a complete reduction in complement mediated lysis (CMCL) (Boyd et al., (1996) *Mol. Immunol.* 32:1311-1318), while selective removal of sialic acid residues using neuraminidase resulted in no loss of DMCL. Glycosylation of antibodies has also been reported to affect antibody-dependent cellular cytotoxicity (ADCC). In particular, CHO cells with tetracycline-regulated expression of β (1,4)-N-acetylglucosaminyltransferase III (GnTIII), a glycosyltransferase catalyzing formation of bisecting GlcNAc, was reported to have improved ADCC activity (Umana et al. (1999) *Mature Biotech.* 17:176-180).

Glycosylation of antibodies is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-acetylglucosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

Glycosylation variants of antibodies are variants in which the glycosylation pattern of an antibody is altered. By altering is meant deleting one or more carbohydrate moieties found in the antibody, adding one or more carbohydrate moieties to the antibody, changing the composition of glycosylation (glycosylation pattern), the extent of glycosylation, etc.

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Addition of glycosylation sites to the antibody is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the original antibody (for O-linked glycosylation sites). Similarly, removal of glycosylation sites can be accomplished by amino acid alteration within the native glycosylation sites of the antibody.

The amino acid sequence is usually altered by altering the underlying nucleic acid sequence. These methods include, but are not limited to, isolation from a natural source (in the case of naturally-occurring amino acid sequence variants) or preparation by oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared variant or a non-variant version of the antibody.

The glycosylation (including glycosylation pattern) of antibodies may also be altered without altering the amino acid sequence or the underlying nucleotide sequence. Glycosylation largely depends on the host cell used to express the antibody. Since the cell type used for expression of recombinant glycoproteins, e.g., antibodies, as potential therapeutics is rarely the native cell, significant variations in the glycosylation pattern of the antibodies can be expected. See, e.g., Hse et al., (1997) *J. Biol. Chem.* 272:9062-9070. In addition to the choice of host cells, factors which affect glycosylation during recombinant production of antibodies include growth mode, media formulation, culture density, oxygenation, pH, purification schemes and the like. Various methods have been proposed to alter the glycosylation pattern achieved in a particular host organism including introducing or overexpressing certain enzymes involved in oligosaccharide production (U.S. Pat. Nos. 5,047,335; 5,510,261; 5,278,299). Glycosylation, or certain types of glycosylation, can be enzymatically removed from the glycoprotein, for example using endoglycosidase H (Endo H). In addition, the recombinant host cell can be genetically engineered, e.g., make defective in processing certain types of polysaccharides. These and similar techniques are well known in the art.

The glycosylation structure of antibodies can be readily analyzed by conventional techniques of carbohydrate analysis, including lectin chromatography, NMR, Mass spectrometry, HPLC, GPC, monosaccharide compositional analysis, sequential enzymatic digestion, and HPAEC-PAD, which uses high pH anion exchange chromatography to separate oligosaccharides based on charge. Methods for releasing oligosaccharides for analytical purposes are also known, and include, without limitation, enzymatic treatment (commonly performed using peptide-N-glycosidase F/endo- β -galactosidase), elimination using harsh alkaline environment to release mainly O-linked structures, and chemical methods using anhydrous hydrazine to release both N- and O-linked oligosaccharides.

9.5.2a Screening for Antibody-Drug Conjugates (ADC)

Transgenic animals and cell lines are particularly useful in screening antibody drug conjugates (ADC) that have potential as prophylactic or therapeutic treatments of diseases or disorders involving overexpression of proteins including Lewis Y, CD30, CD40, and CD70. Transgenic animals and cell lines are particularly useful in screening antibody drug conjugates (ADC) that have potential as prophylactic or therapeutic treatments of diseases or disorders involving overexpression of HER2 (U.S. Pat. No. 6,632,979). Screening for a useful ADC may involve administering candidate

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ADC over a range of doses to the transgenic animal, and assaying at various time points for the effect(s) of the ADC on the disease or disorder being evaluated. Alternatively, or additionally, the drug can be administered prior to or simultaneously with exposure to an inducer of the disease, if applicable. Candidate ADC may be screened serially and individually, or in parallel under medium or high-throughput screening format. The rate at which ADC may be screened for utility for prophylactic or therapeutic treatments of diseases or disorders is limited only by the rate of synthesis or screening methodology, including detecting/measuring/analysis of data.

One embodiment is a screening method comprising (a) transplanting cells from a stable renal cell cancer cell line into a non-human animal, (b) administering an ADC drug candidate to the non-human animal and (c) determining the ability of the candidate to inhibit the formation of tumors from the transplanted cell line.

Another embodiment is a screening method comprising (a) contacting cells from a stable Hodgkin's disease cell line with an ADC drug candidate and (b) evaluating the ability of the ADC candidate to block ligand activation of CD40.

Another embodiment is a screening method comprising (a) contacting cells from a stable Hodgkin's disease cell line with an ADC drug candidate and (b) evaluating the ability of the ADC candidate to induce cell death. In one embodiment the ability of the ADC candidate to induce apoptosis is evaluated.

One embodiment is a screening method comprising (a) transplanting cells from a stable cancer cell line into a non-human animal, (b) administering an ADC drug candidate to the non-human animal and (c) determining the ability of the candidate to inhibit the formation of tumors from the transplanted cell line. The invention also concerns a method of screening ADC candidates for the treatment of a disease or disorder characterized by the overexpression of HER2 comprising (a) contacting cells from a stable breast cancer cell line with a drug candidate and (b) evaluating the ability of the ADC candidate to inhibit the growth of the stable cell line.

Another embodiment is a screening method comprising (a) contacting cells from a stable cancer cell line with an ADC drug candidate and (b) evaluating the ability of the ADC candidate to block ligand activation of HER2. In one embodiment the ability of the ADC candidate to block heregulin binding is evaluated. In another embodiment the ability of the ADC candidate to block ligand-stimulated tyrosine phosphorylation is evaluated.

Another embodiment is a screening method comprising (a) contacting cells from a stable cancer cell line with an ADC drug candidate and (b) evaluating the ability of the ADC candidate to induce cell death. In one embodiment the ability of the ADC candidate to induce apoptosis is evaluated.

Another embodiment is a screening method comprising (a) administering an ADC drug candidate to a transgenic non-human mammal that overexpresses in its mammary gland cells a native human HER2 protein or a fragment thereof, wherein such transgenic mammal has stably integrated into its genome a nucleic acid sequence encoding a native human HER2 protein or a fragment thereof having the biological activity of native human HER2, operably linked to transcriptional regulatory sequences directing its expression to the mammary gland, and develops a mammary tumor not responding or poorly responding to anti-HER2 antibody treatment, or to a non-human mammal bearing a tumor transplanted from said transgenic non-human mammal; and

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(b) evaluating the effect of the ADC candidate on the target disease or disorder. Without limitations, the disease or disorder may be a HER2-overexpressing cancer, such as breast, ovarian, stomach, endometrial, salivary gland, lung, kidney, colon, thyroid, pancreatic and bladder cancer. The cancer preferably is breast cancer which expressed HER2 in at least about 500,000 copies per cell, more preferably at least about 2,000,000 copies per cell. ADC drug candidates may, for example, be evaluated for their ability to induce cell death and/or apoptosis, using assay methods well known in the art and described hereinafter.

In one embodiment, candidate ADC are screened by being administered to the transgenic animal over a range of doses, and evaluating the animal's physiological response to the compounds over time. Administration may be oral, or by suitable injection, depending on the chemical nature of the compound being evaluated. In some cases, it may be appropriate to administer the compound in conjunction with co-factors that would enhance the efficacy of the compound. If cell lines derived from the subject transgenic animals are used to screen for compounds useful in treating various disorders, the test compounds are added to the cell culture medium at an appropriate time, and the cellular response to the compound is evaluated over time using the appropriate biochemical and/or histological assays. In some cases, it may be appropriate to apply the compound of interest to the culture medium in conjunction with co-factors that would enhance the efficacy of the compound.

Thus, provided herein are assays for identifying ADC which specifically target and bind a target protein, the presence of which is correlated with abnormal cellular function, and in the pathogenesis of cellular proliferation and/or differentiation that is causally related to the development of tumors.

To identify an ADC which blocks ligand activation of an ErbB (e.g., ErbB2) receptor, the ability of the compound to block ErbB ligand binding to cells expressing the ErbB (ErbB2) receptor (e.g., in conjugation with another ErbB receptor with which the ErbB receptor of interest forms an ErbB hetero-oligomer) may be determined. For example, cells isolated from the transgenic animal overexpressing HER2 and transfected to express another ErbB receptor (with which HER2 forms hetero-oligomer) may be incubated, i.e. culturing, with the ADC and then exposed to labeled ErbB ligand. The ability of the compound to block ligand binding to the ErbB receptor in the ErbB hetero-oligomer may then be evaluated.

For example, inhibition of heregulin (HRG) binding to breast tumor cell lines, overexpressing HER2 and established from the transgenic non-human mammals (e.g., mice) herein, by the candidate ADC may be performed using monolayer cultures on ice in a 24-well-plate format. Anti-ErbB2 monoclonal antibodies may be added to each well and incubated for 30 minutes. ¹²⁵I-labeled rHRGβ1 (25,000 cpm) may then be added, and the incubation may be continued for 4 to 16 hours. Dose response curves may be prepared and an IC₅₀ value (cytotoxic activity) may be calculated for the compound of interest.

Alternatively, or additionally, the ability of an ADC to block ErbB ligand-stimulated tyrosine phosphorylation of an ErbB receptor present in an ErbB hetero-oligomer may be assessed. For example, cell lines established from the transgenic animals herein may be incubated with a test ADC and then assayed for ErbB ligand-dependent tyrosine phosphorylation activity using an anti-phosphotyrosine monoclonal antibody (which is optionally conjugated with a detectable label). The kinase receptor activation assay described in U.S.

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Pat. No. 5,766,863 is also available for determining ErbB receptor activation and blocking of that activity by the compound.

In one embodiment, one may screen for ADC which inhibit HRG stimulation of p180 tyrosine phosphorylation in MCF7 cells essentially as described below. For example, a cell line established from a HER2-transgenic animal may be plated in 24-well plates and the compound may be added to each well and incubated for 30 minutes at room temperature; then rHRG β _{1,177-244} may be added to each well to a final concentration of 0.2 nM, and the incubation may be continued for about 8 minutes. Media may be aspirated from each well, and reactions may be stopped by the addition of 100 μ l of SDS sample buffer (5% SDS, 25 mM DTT, and 25 mM Tris-HCl, pH 6.8). Each sample (25 μ l) may be electrophoresed on a 4-12% gradient gel (Novex) and then electrophoretically transferred to polyvinylidene difluoride membrane. Antiphosphotyrosine (at 1 μ g/ml) immunoblots may be developed, and the intensity of the predominant reactive band at M_r~180,000 may be quantified by reflectance densitometry. An alternate method to evaluate inhibition of receptor phosphorylation is the KIRA (kinase receptor activation) assay of Sadick et al. (1998) *Jour. of Pharm. and Biomed. Anal.* Some of the well established monoclonal antibodies against HER2 that are known to inhibit HRG stimulation of p180 tyrosine phosphorylation can be used as positive control in this assay. A dose-response curve for inhibition of HRG stimulation of p180 tyrosine phosphorylation as determined by reflectance densitometry may be prepared and an IC₅₀ for the compound of interest may be calculated.

One may also assess the growth inhibitory effects of a test ADC on cell lines derived from a HER2-transgenic animal, e.g., essentially as described in Schaefer et al. (1997) *Oncogene* 15:1385-1394. According to this assay, the cells may be treated with a test compound at various concentrations for 4 days and stained with crystal violet or the redox dye Alamar Blue. Incubation with the compound may show a growth inhibitory effect on this cell line similar to that displayed by monoclonal antibody 2C₄ on MDA-MB-175 cells (Schaefer et al., supra). In a further embodiment, exogenous HRG will not significantly reverse this inhibition.

To identify growth inhibitory compounds that specifically target an antigen of interest, one may screen for compounds which inhibit the growth of cancer cells overexpressing antigen of interest derived from transgenic animals, the assay described in U.S. Pat. No. 5,677,171 can be performed. According to this assay, cancer cells overexpressing the antigen of interest are grown in a 1:1 mixture of F12 and DMEM medium supplemented with 10% fetal bovine serum, glutamine and penicillin streptomycin. The cells are plated at 20,000 cells in a 35 mm cell culture dish (2 mls/35 mm dish) and the test compound is added at various concentrations. After six days, the number of cells, compared to untreated cells is counted using an electronic COULTER™ cell counter. Those compounds which inhibit cell growth by about 20-100% or about 50-100% may be selected as growth inhibitory compounds.

To select for compounds which induce cell death, loss of membrane integrity as indicated by, e.g., PI, trypan blue or 7AAD uptake may be assessed relative to control. The PI uptake assay uses cells isolated from the tumor tissue of interest of a transgenic animal. According to this assay, the cells are cultured in Dulbecco's Modified Eagle Medium (D-MEM):Ham's F-12 (50:50) supplemented with 10% heat-inactivated FBS (Hyclone) and 2 mM L-glutamine.

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Thus, the assay is performed in the absence of complement and immune effector cells. The cells are seeded at a density of 3×10^6 per dish in 100×20 mm dishes and allowed to attach overnight. The medium is then removed and replaced with fresh medium alone or medium containing various concentrations of the compound. The cells are incubated for a 3-day time period. Following each treatment, monolayers are washed with PBS and detached by trypsinization. Cells are then centrifuged at 1200 rpm for 5 minutes at 4° C., the pellet resuspended in 3 ml cold Ca²⁺ binding buffer (10 mM Hepes, pH 7.4, 140 mM NaCl, 2.5 mM CaCl₂) and aliquoted into 35 mm strainer-capped 12×75 mm tubes (1 ml per tube, 3 tubes per treatment group) for removal of cell clumps. Tubes then receive PI (10 μ g/ml). Samples may be analyzed using a FACSCAN™ flow cytometer and FACSCONVERT™ CellQuest software (Becton Dickinson). Those compounds which induce statistically significant levels of cell death as determined by PI uptake may be selected as cell death-inducing compounds.

In order to select for compounds which induce apoptosis, an annexin binding assay using cells established from the tumor tissue of interest of the transgenic animal is performed. The cells are cultured and seeded in dishes as discussed in the preceding paragraph. The medium is then removed and replaced with fresh medium alone or medium containing 10 μ g/ml of the antibody drug conjugate (ADC). Following a three-day incubation period, monolayers are washed with PBS and detached by trypsinization. Cells are then centrifuged, resuspended in Ca²⁺ binding buffer and aliquoted into tubes as discussed above for the cell death assay. Tubes then receive labeled annexin (e.g., annexin V-FITC) (1 μ g/ml). Samples may be analyzed using a FACSCAN™ flow cytometer and FACSCONVERT™ CellQuest software (Becton Dickinson). Those compounds which induce statistically significant levels of annexin binding relative to control are selected as apoptosis-inducing compounds.

9.5.3 In Vitro Cell Proliferation Assays

Generally, the cytotoxic or cytostatic activity of an antibody drug conjugate (ADC) is measured by: exposing mammalian cells having receptor proteins to the antibody of the ADC in a cell culture medium; culturing the cells for a period from about 6 hours to about 5 days; and measuring cell viability. Cell-based in vitro assays were used to measure viability (proliferation), cytotoxicity, and induction of apoptosis (caspase activation) of the ADC of the invention.

The in vitro potency of antibody drug conjugates was measured by a cell proliferation assay (Example 18, FIGS. 7-10). The CellTiter-Glo® Luminescent Cell Viability Assay is a commercially available (Promega Corp., Madison, Wis.), homogeneous assay method based on the recombinant expression of *Coleoptera* luciferase (U.S. Pat. Nos. 5,583,024; 5,674,713 and 5,700,670). This cell proliferation assay determines the number of viable cells in culture based on quantitation of the ATP present, an indicator of metabolically active cells (Crouch et al. (1993) *J. Immunol. Meth.* 160:81-88, U.S. Pat. No. 6,602,677). The CellTiter-Glo® Assay was conducted in 96 well format, making it amenable to automated high-throughput screening (HTS) (Cree et al. (1995) *AntiCancer Drugs* 6:398-404). The homogeneous assay procedure involves adding the single reagent (CellTiter-Glo® Reagent) directly to cells cultured in serum-supplemented medium. Cell washing, removal of medium and multiple pipetting steps are not required. The system detects as few as 15 cells/well in a 384-well format in 10 minutes after adding reagent and mixing. The cells may be treated continuously with ADC, or they may be

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treated and separated from ADC. Generally, cells treated briefly, i.e. 3 hours, showed the same potency effects as continuously treated cells.

The homogeneous “add-mix-measure” format results in cell lysis and generation of a luminescent signal proportional to the amount of ATP present. The amount of ATP is directly proportional to the number of cells present in culture. The CellTiterGlo® Assay generates a “glow-type” luminescent signal, produced by the luciferase reaction, which has a half-life generally greater than five hours, depending on cell type and medium used (FIG. 24). Viable cells are reflected in relative luminescence units (RLU). The substrate, Beetle Luciferin, is oxidatively decarboxylated by recombinant firefly luciferase with concomitant conversion of ATP to AMP and generation of photons. The extended half-life eliminates the need to use reagent injectors and provides flexibility for continuous or batch mode processing of multiple plates. This cell proliferation assay can be used with various multiwell formats, e.g., 96 or 384 well format. Data can be recorded by luminometer or CCD camera imaging device. The luminescence output is presented as relative light units (RLU), measured over time.

The anti-proliferative effects of antibody drug conjugates were measured by the cell proliferation, in vitro cell killing assay above against four different breast tumor cell lines (FIGS. 7-10). IC₅₀ values were established for SK-BR-3 and BT-474 which are known to over express HER2 receptor protein. Table 2a shows the potency (IC₅₀) measurements of exemplary antibody drug conjugates in the cell proliferation assay against SK-BR-3 cells. Table 2b shows the potency (IC₅₀) measurements of exemplary antibody drug conjugates in the cell proliferation assay against BT-474 cells.

Antibody drug conjugates: Trastuzumab-MC-vc-PAB-MMAF, 3.8 MMAF/Ab; Trastuzumab-MC-(N-Me)vc-PAB-MMAF, 3.9 MMAF/Ab; Trastuzumab-MC-MMAF, 4.1 MMAF/Ab; Trastuzumab-MC-vc-PAB-MMAE, 4.1 MMAE/Ab; Trastuzumab-MC-vc-PAB-MMAE, 3.3 MMAE/Ab; and Trastuzumab-MC-vc-PAB-MMAF, 3.7 MMAF/Ab did not inhibit the proliferation of MCF-7 cells (FIG. 9).

Antibody drug conjugates: Trastuzumab-MC-vc-PAB-MMAE, 4.1 MMAE/Ab; Trastuzumab-MC-vc-PAB-MMAE, 3.3 MMAE/Ab; Trastuzumab-MC-vc-PAB-MMAF, 3.7 MMAF/Ab; Trastuzumab-MC-vc-PAB-MMAF, 3.8 MMAF/Ab; Trastuzumab-MC-(N-Me)vc-PAB-MMAF, 3.9 MMAF/Ab; and Trastuzumab-MC-MMAF, 4.1 MMAF/Ab did not inhibit the proliferation of MDA-MB-468 cells (FIG. 10).

MCF-7 and MDA-MB-468 cells do not overexpress HER2 receptor protein. The anti-HER2 antibody drug conjugates of the invention therefore show selectivity for inhibition of cells which express HER2.

TABLE 2a

SK-BR-3 cells	
Antibody Drug Conjugate H = trastuzumab linked via a cysteine [cys] except where noted	IC ₅₀ (μg ADC/ml)
H-MC-MMAF, 4.1 MMAF/Ab	0.008
H-MC-MMAF, 4.8 MMAF/Ab	0.002
H-MC-vc-PAB-MMAE,	0.007
H-MC-vc-PAB-MMAE	0.015
H-MC-vc-PAB-MMAF, 3.8 MMAF/Ab	0.0035-0.01
H-MC-vc-PAB-MMAF, 4.4 MMAF/Ab	0.006-0.007
H-MC-vc-PAB-MMAF, 4.8 MMAF/Ab	0.006
H-MC-(N—Me)vc-PAB-MMAF, 3.9 MMAF/Ab	0.0035

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TABLE 2a-continued

SK-BR-3 cells	
Antibody Drug Conjugate H = trastuzumab linked via a cysteine [cys] except where noted	IC ₅₀ (μg ADC/ml)
H-MC-MMAF, 4.1 MMAF/Ab	0.0035
H-MC-vc-PAB-MMAE, 4.1 MMAE/Ab	0.010
H-MC-vc-PAB-MMAF, 3.8 MMAF/Ab	0.007
H-MC-vc-PAB-MMAE, 4.1 MMAE/Ab	0.015
H-MC-vc-PAB-MMAF, 3.7 MMAF/Ab.	0.010
H-MC-vc-PAB-MMAE, 7.5 MMAE/Ab	0.0025
H-MC-MMAE, 8.8 MMAE/Ab	0.018
H-MC-MMAE, 4.6 MMAE/Ab	0.05
H-MC-(L)val-(L)cit-PAB-MMAE, 8.7 MMAE/Ab	0.0003
H-MC-(D)val-(D)cit-PAB-MMAE, 8.2 MMAE/Ab	0.02
H-MC-(D)val-(L)cit-PAB-MMAE, 8.4 MMAE/Ab	0.0015
H-MC-(D)val-(L)cit-PAB-MMAE, 3.2 MMAE/Ab	0.003
H-Trastuzumab	0.083
H-vc-MMAE, linked via a lysine [lys]	0.002
H-phe-lys-MMAE, linked via a lysine [lys]	0.0015
4D5-Fe8-MC-vc-PAB-MMAF, 4.4 MMAF/Ab	0.004
Hg-MC-vc-PAB-MMAF, 4.1 MMAF/Ab	0.01
7C2-MC-vc-PAB-MMAF, 4.0 MMAF/Ab	0.01
4D5 Fab-MC-vc-PAB-MMAF, 1.5 MMAF/Ab	0.02
Anti-TF Fab-MC-vc-PAB-MMAE*	—

TABLE 2b

BT474 cells	
Antibody Drug Conjugate H = trastuzumab linked via a cysteine [cys]	IC ₅₀ (μg ADC/ml)
H-MC-MMAF, 4.1 MMAF/Ab	0.008
H-MC-MMAF, 4.8 MMAF/Ab	0.002
H-MC-vc-PAB-MMAE, 4.1 MMAE/Ab	0.015
H-MC-vc-PAB-MMAF, 3.8 MMAF/Ab	0.02-0.05
H-MC-vc-PAB-MMAF, 4.4 MMAF/Ab	0.01
H-MC-vc-PAB-MMAF, 4.8 MMAF/Ab	0.01
H-MC-vc-PAB-MMAE, 3.3 MMAE/Ab	0.02
H-MC-vc-PAB-MMAF, 3.7 MMAF/Ab.	0.02
H-MC-vc-PAB-MMAF, 3.8 MMAF/Ab	0.015
H-MC-(N—Me)vc-PAB-MMAF, 3.9 MMAF/Ab	0.010
H-MC-MMAF, 4.1 MMAF/Ab	0.00015
H-MC-vc-PAB-MMAE, 7.5 MMAE/Ab	0.0025
H-MC-MMAE, 8.8 MMAE/Ab	0.04
H-MC-MMAE, 4.6 MMAE/Ab	0.07
4D5-Fe8-MC-vc-PAB-MMAF, 4.4 MMAF/Ab	0.008
Hg-MC-vc-PAB-MMAF, 4.1 MMAF/Ab	0.01
7C2-MC-vc-PAB-MMAF, 4.0 MMAF/Ab	0.015
4D5 Fab-MC-vc-PAB-MMAF, 1.5 MMAF/Ab	0.04
Anti-TF Fab-MC-vc-PAB-MMAE*	—

H = trastuzumab
7C2 = anti-HER2 murine antibody which binds a different epitope than trastuzumab.
Fe8 = mutant that does not bind to FeRn
Hg = “Hingeless” full-length humanized 4D5, with heavy chain hinge cysteines mutated to serines. Expressed in *E. coli* (therefore non-glycosylated.)
Anti-TF Fab = anti-tissue factor antibody fragment
*activity against MDA-MB-468 cells

In a surprising and unexpected discovery, the in vitro cell proliferation activity results of the ADC in Tables 2a and 2b show generally that ADC with a low average number of drug moieties per antibody showed efficacy, e.g., IC₅₀ < 0.1 μg ADC/ml. The results suggest that at least for trastuzumab ADC, the optimal ratio of drug moieties per antibody may be less than 8, and may be about 2 to about 5.

9.5.4 In Vivo Plasma Clearance and Stability

Pharmacokinetic plasma clearance and stability of ADC were investigated in rats and cynomolgus monkeys. Plasma concentration was measured over time. Table 2c shows

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pharmacokinetic data of antibody drug conjugates and other dosed samples in rats. Rats are a non-specific model for

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ErbB receptor antibodies, since the rat is not known to express HER2 receptor proteins.

TABLE 2c

Pharmacokinetics in Rats					
H = trastuzumab linked via a cysteine					
[cys] except where noted					
2 mg/kg dose except where noted					
Sample dose mg/kg	AUCinf day* µg/mL	CL mL/day/kg	Cmax µg/mL	T ½ Term. days	% Conj.
H-MC-vc-PAB-MMAE (Total Ab)	78.6	26.3	39.5	5.80	40.6
H-MC-vc-PAB-MMAE (Conj.)	31.1	64.4	33.2	3.00	
H-MC-vc-PAB-MMAF (Total Ab)	170	12.0	47.9	8.4	50.0
H-MC-vc-PAB-MMAF (Conj.)	83.9	24.0	44.7	4.01	
H-MC-MMAE (Total Ab)	279	18.9	79.6	7.65	33
H-MC-MMAE (Conj.)	90.6	62.9	62.9	4.46	
5 mg/kg					
H-MC-MMAF (Total Ab)	299	6.74	49.1	11.6	37
H-MC-MMAF (Conj.)	110	18.26	50.2	4.54	
H-MC-vc-MMAF, wo/PAB, (Total Ab)	306	6.6	78.7	11.9	19.6
H-MC-vc-MMAF, wo/PAB, (Conj.)	59.9	33.4	82.8	2.1	
H-Me-vc-PAB-MMAF (Total Ab)	186	10.8	46.9	8.3	45.3
H-Me-vc-PAB-MMAF (Conj.)	84.0	23.8	49.6	4.3	
H-Me-vc-PAB-MMAE (Total Ab)	135	15.0	44.9	11.2	23.8
H-Me-vc-PAB-MMAE (Conj.)	31.9	63.8	45.2	3.0	
H-MC-vc-MMAF, wo/PAB, (Total Ab)	306	6.6	78.7	11.9	19.6
H-MC-vc-MMAF, wo/PAB, (Conj.)	59.9	33.4	82.8	2.1	
H-MC-(D)val-(L)cit-PAB-MMAE (Total Ab)	107	19.2	30.6	9.6	38.1
H-MC-(D)val-(L)cit-PAB-MMAE (Conj.)	40	50.4	33.7	3.98	
H-MC-(Me)-vc-PAB-MMAE, Total Ab	135.1	15.0	44.9	11.2	23.8
H-MC-(Me)-vc-PAB-MMAE, Conj.	31.9	63.8	45.2	2.96	
H-MC-(D)val-(D)cit-PAB-MMAE, Total Ab	88.2	22.8	33.8	10.5	38.3
H-MC-(D)val-(D)cit-PAB-MMAE, Conj.	33.6	59.8	36.0	4.43	
H-MC-vc-PAB-MMAE, Total Ab	78.6	26.3	39.5	5.8	40.6
H-MC-vc-PAB-MMAE, Conj.	31.1	64.4	33.2	3.00	
H linked to MC by lysine [lys]					
MMAF 200 µg/kg	0.99	204	280	0.224	—
MMAE 206 µg/kg	3.71	62.6	649	0.743	—
HER F(ab') ₂ -MC-vc-MMAE, Total Ab	9.3	217	34.4	0.35	95
HER F(ab') ₂ -MC-vc-MMAE, Conj.	8.8	227	36.9	0.29	
4D5-H-Fab-MC-vc-MMAF, Total Ab	43.8	46.2	38.5	1.49	68
4D5-H-Fab-MC-vc-MMAF, Conj.	29.9	68.1	34.1	1.12	
4D5-H-Fab-MC-vc-MMAE, Total Ab	71.5	70.3	108	1.18	59
4D5-H-Fab-MC-vc-MMAE, Conj.	42.2	118.9	114	0.74	
4D5-H-Fab	93.4	53.9	133	1.08	—
H-MC-vc-PAB-MMAF, Total Ab	170	12.03	47.9	8.44	49.5
H-MC-vc-PAB-MMAF, Conj.	83.9	23.96	44.7	4.01	

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TABLE 2c-continued

Pharmacokinetics in Rats H = trastuzumab linked via a cysteine [cys] except where noted 2 mg/kg dose except where noted					
Sample dose mg/kg	AUCinf day* µg/mL	CL mL/day/kg	Cmax µg/mL	T 1/2 Term. days	% Conj.
H-MC-vc-PAB-MMAF- DMAEA, Total Ab	211	9.8	39.8	8.53	34.3
H-MC-vc-PAB-MMAF- DMAEA, Conj.	71.5	28.2	38.8	3.64	
H-MC-vc-PAB-MMAF-TEG, Total Ab	209	9.75	53.2	8.32	29.7
H-MC-vc-PAB-MMAF-TEG, Conj.	63.4	31.8	34.9	4.36	

AUC inf is the area under the plasma concentration-time curve from time of dosing to infinity and is a measure of the total exposure to the measured entity (drug, ADC). CL is defined as the volume of plasma cleared of the measured entity in unit time and is expressed by normalizing to body weight. T1/2 term is the half-life of the drug in the body measured during its elimination phase. The % Conj. term is the relative amount of ADC compared to total antibody detected, by separate ELISA immunoaffinity tests ("Analytical Methods for Biotechnology Products", Ferraiolo et al, p 85-98 in Pharmacokinetics of Drugs (1994) P. G. Welling and L. P. Balant, Eds., Handbook of Experimental Pharmacology, Vol. 110, Springer-Verlag. The % Conj. calculation is simply AUCinf of ADC+ AUCinf total Ab, and is a general indicator of linker stability, although other factors and mechanisms may be in effect.

FIG. 11 shows a graph of a plasma concentration clearance study after administration of the antibody drug conjugates: H-MC-vc-PAB-MMAF-TEG and H-MC-vc-PAB-MMAF to Sprague-Dawley rats. Concentrations of total antibody and ADC were measured over time.

FIG. 12 shows a graph of a two stage plasma concentration clearance study where ADC was administered at different dosages and concentrations of total antibody and ADC were measured over time.

9.5.4a In Vivo Efficacy

The in vivo efficacy of the ADC of the invention was measured by a high expressing HER2 transgenic explant mouse model. An allograft was propagated from the Fo5 mmtv transgenic mouse which does not respond to, or responds poorly to, HERCEPTIN® therapy. Subjects were treated once with ADC and monitored over 3-6 weeks to measure the time to tumor doubling, log cell kill, and tumor shrinkage. Follow up dose-response and multi-dose experiments were conducted.

Tumors arise readily in transgenic mice that express a mutationally activated form of neu, the rat homolog of HER2, but the HER2 that is overexpressed in breast cancers is not mutated and tumor formation is much less robust in transgenic mice that overexpress nonmutated HER2 (Webster et al. (1994) Semin. Cancer Biol. 5:69-76).

To improve tumor formation with nonmutated HER2, transgenic mice were produced using a HER2 cDNA plasmid in which an upstream ATG was deleted in order to prevent initiation of translation at such upstream ATG codons, which would otherwise reduce the frequency of translation initiation from the downstream authentic initiation codon of HER2 (for example, see Child et al. (1999) J. Biol. Chem. 274: 24335-24341). Additionally, a chimeric intron was added to the 5' end, which should also enhance the level of expression as reported earlier (Neuberger and

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Williams (1988) Nucleic Acids Res. 16: 6713; Buchman and Berg (1988) Mol. Cell. Biol. 8:4395; Brinster et al. (1988) Proc. Natl. Acad. Sci. USA 85:836). The chimeric intron was derived from a Promega vector, pCI-neo mammalian expression vector (bp 890-1022). The cDNA 3'-end is flanked by human growth hormone exons 4 and 5, and polyadenylation sequences. Moreover, FVB mice were used because this strain is more susceptible to tumor development. The promoter from MMTV-LTR was used to ensure tissue-specific HER2 expression in the mammary gland. Animals were fed the AIN 76A diet in order to increase susceptibility to tumor formation (Rao et al. (1997) Breast Cancer Res. and Treatment 45:149-158).

TABLE 2d

Tumor measurements in allograft mouse model—MMTV-HER2 Fo5 Mammary Tumor, athymic nude mice single dose at day 1 (T = 0) except where noted H = trastuzumab linked via a cysteine [cys] except where noted							
Sample Drugs per antibody	Dose	Ti	PR	CR	Tumor doubling time (days)	Mean log cell kill	
Vehicle					2-5	0	
H-MC-vc- PAB-MMAE	1250 µg/m ²	5/	4/7	0/7	18	1.5	
8.7 MMEA/Ab		5					
H-MC-vc- PAB-MMAF	555 µg/m ²	2/	2/7	5/7	69	6.6	
3.8 MMAF/Ab		5					
H-MC(Me)-vc- PAB-MMAF					>50	6.4	
H-MC-MMAF	9.2 mg/kg	7/	6/7	0/7	63	9	
4.8 MMAF/Ab		7					
	550 µg/m ² at 0, 7, 14 and 21 days						
H-MC-MMAF	14 mg/kg Ab	5/	5/7	2/7	>63		
4.8 MMAF/Ab	840 µg/m ² at 0, 7, 14 and 21 days	5					
H-MC-vc- PAB-MMAF	3.5 mg/kg	5/	1/7	3/7	>36		
5.9 MMAF/Ab	Ab	6					
	300 µg/m ² at 0, 21, and 42 days						
H-MC-vc- PAB-MMAF	4.9 mg/kg	4/	2/7	5/7	>90		
5.9 MMAF/Ab	Ab	7					
	425 µg/m ² at 0, 21, and 42 days						

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TABLE 2d-continued

Tumor measurements in allograft mouse model—MMTV-HER2 Fo5 Mammary Tumor, athymic nude mice single dose at day 1 (T = 0) except where noted H = trastuzumab linked via a cysteine [cys] except where noted						
Sample Drugs per antibody	Dose	Ti	PR	CR	Tumor doubl- ing time (days)	Mean log cell kill
H-MC-vc- PAB-MMAF	6.4 mg/kg	3/	1/7	6/7	>90	
5.9 MMAF/Ab	550 µg/m ² at 0, 21, and 42 days	6				
H-(L)val- (L)cit-MMAE	10 mg/kg	7/	1/7	0/7	15.2	1.1
8.7 MMAE/Ab		7				
H-MC-MMAE	10 mg/kg	7/	0/7	0/7	4	0.1
4.6 MMAE/Ab		7				
H-(D)val- (D)cit-MMAE	10 mg/kg	7/	0/7	0/7	3	
4.2 MMAE/Ab		7				
H-(D)val- (D)cit-MMAE	13 mg/kg	7/	0/7	0/7	9	0.6
3.2 MMAE/Ab		7				
H-MC(Me)-vc- MMAE	13 mg/kg	7/	3/7	0/7	17	1.2
3.0 MMAE/Ab		7				
H-(L)val- (D)cit-MMAE	12 mg/kg	7/	0/7	0/7	5	0.2
3.5 MMAE/Ab		7				
H-vc-MMAE	10 mg/kg	7/			17	
8.7 MMAE/Ab		7				
H-cys-vc- MMAF	1 mg/kg	7/			3	
3.8 MMAF/Ab		7				
H-cys-vc- -MMAF	3 mg/kg	7/			>17	
3.8 MMAF/Ab		7				
H-cys-vc- MMAF	10 mg/kg	4/	4/7	3/7	>17	
3.8 MMAF/Ab		7				
H-MC-vc- MMAF-TEG	10 mg/kg	3/	1/7	6/7	81	7.8
4 MMAF/Ab		6				
H-MC-vc- MMAF-TEG	10 mg/kg	0/	0/7	7/7	81	7.9
4 MMAF/Ab	q3wk x 3	5				
H-vc-MMAF (lot 1)	10 mg/kg	4/	2/8	5/8		
H-vc-MMAF (lot 2)	10 mg/kg	7/	1/8	1/8		
H-MC- MMAF	10 mg/kg	8/	1/8	0/8	18	
550 µg/m ²		8				
H-(Me)-vc- MMAF	10 mg/kg	3/	2/8	5/8		
7		7				
H-vc-MMAE	3.7 mg/kg at	6/	0/7	1/7	17	2.3
7.5 MMAE/ Ab	0, 7, 14, 21, 28 days	6				
H-vc-MMAE	7.5 mg/kg at	5/	3/7	3/7	69	10
7.5 MMAE/ Ab	0, 7, 14, 21, 28 days	7				
anti IL8-vc- MMAE	7.5 mg/kg at	7/	0/7	0/7	5	0.5
7.5 MMAE/Ab	0, 7, 14, 21, 28 days	7				
anti IL8-vc- MMAE	3.7 mg/kg at	6/	0/7	0/7	3	0.2
7.5 MMAE/Ab	0, 7, 14, 21, 28 days	6				
H-fk-MMAE	7.5 mg/kg at	7/	1/7	0/7	31	4.4
7.5 MMAE/ Ab	0, 7, 14, 21, 28 days	7				
H-fk-MMAE	3/7 mg/kg at	7/	0/7	0/7	8.3	0.9
7.5 MMAE/ Ab	0, 7, 14, 21, 28 days	7				
anti IL8-fk -MMAE	7.5 mg/kg at	7/	0/7	0/7	6	0.5
7.5 MMAE/Ab	0, 7, 14, 21, 28 days	7				

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TABLE 2d-continued

Tumor measurements in allograft mouse model—MMTV-HER2 Fo5 Mammary Tumor, athymic nude mice single dose at day 1 (T = 0) except where noted H = trastuzumab linked via a cysteine [cys] except where noted						
Sample Drugs per antibody	Dose	Ti	PR	CR	Tumor doubl- ing time (days)	Mean log cell kill
anti IL8-fk- MMAE	3.7 mg/kg at	7/	0/7	0/7	3	0.1
0, 7, 14, 21, 28 days		7				
7.5 MMAE/Ab	7.5 mg/kg at	7/	0/7	0/7	5	0.4
Trastuzumab	0, 7, 14, 21, 28 days	7				
H-vc-MMAE	10 mg/kg	6/	3/6	0/6	15	1.3
8.7 MMAE/Ab	1250 µg/m ²	6				
H-vc-MMAE	10 mg/kg	7/	5/7		>19	
1250 µg/m ²		7				
at 0, 7, and 14 days						
H-vc-MMAE	3 mg/kg at	7/			8	
0, 7, and 14 days		7				
H-vc-MMAE	1 mg/kg at	7/			7	
0, 7, and 14 days		7				
H-vc-MMAF	10 mg/kg	8/	5/8		>21	
8		8				
H-vc-MMAF	10 mg/kg at	4/	4/7	3/7	>21	
0, 7, and 14 days		7				
H-vc-MMAF	3 mg/kg at	7/			6	
0, 7, and 14 days		7				
Trastuzumab	10 mg/kg at	8/			3	
0 and 7 days		8				
Hg-MC-vc- PAB-MMAF	10 mk/kg at	6/	3/8	5/8	56	5.1
0 days		7				
4.1 MMAF/Ab	10 mg/kg at	7/	6/8	0/8	25	2.1
Fc8-MC-vc- PAB-MMAF	0 days	7				
4.4 MMAF/Ab	10 mg/kg at	5/	6/8	1/8	41	3.7
7C2-MC-vc- PAB-MMAF	0 days	6				
4 MMAF/Ab	10 mg/kg at	3/	3/8	5/8	62	5.7
H-MC-vc- PAB-MMAF	0 days	8				
5.9 MMAF/Ab						
2H9-MC-vc- PAB-MMAE		9/			>14 days	
9		9				
2H9-MC-vc- PAB-MMAF		9/			>14 days	
9		9				
11D10-vc- PAB-MMAE		9/			>14 days	
9		9				
11D10-vc- PAB-MMAF		9/			11 days	
9		9				

7C2 = anti-HER2 murine antibody which binds a different epitope than trastuzumab.

Fc8 = mutant that does not bind to FcRn

Hg = "Hingeless" full-length humanized 4D5, with heavy chain hinge cysteines mutated to serines. Expressed in *E. coli* (therefore non-glycosylated.)

2H9 = Anti-EphB2R

11D10 = Anti-0772P

The term Ti is the number of animals in the study group with tumor at T=0+total animals in group. The term PR is the number of animals attaining partial remission of tumor+animals with tumor at T=0 in group. The term CR is the number of animals attaining complete remission of tumor+animals with tumor at T=0 in group. The term Log cell kill is the time in days for the tumor volume to double—the time in days for the control tumor volume to double divided by 3.32×time for tumor volume to double in control animals (dosed with Vehicle). The log-cell-kill calculation takes into account tumor growth delay resulting

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from treatment and tumor volume doubling time of the control group. Anti-tumor activity of ADC is classified with log-cell-kill values of:

++++	≥3.4	(highly active)
+++ =	2.5-3.4	
++ =	1.7-2.4	
+ =	1.0-1.6	
inactive =	0	

FIG. 13 shows the mean tumor volume change over time in athymic nude mice with MMTV-HER2 Fo5 Mammary tumor allografts dosed on Day 0 with: Vehicle, Trastuzumab-MC-vc-PAB-MMAE (1250 $\mu\text{g}/\text{m}^2$) and Trastuzumab-MC-vc-PAB-MMAF (555 $\mu\text{g}/\text{m}^2$). (H=Trastuzumab). The growth of tumors was retarded by treatment with ADC as compared to control (Vehicle) level of growth. FIG. 14 shows the mean tumor volume change over time in athymic nude mice with MMTV-HER2 Fo5 Mammary tumor allografts dosed on Day 0 with 10 mg/kg (660 $\mu\text{g}/\text{m}^2$) of Trastuzumab-MC-MMAE and 1250 $\mu\text{g}/\text{m}^2$ Trastuzumab-MC-vc-PAB-MMAE. FIG. 15 shows the mean tumor volume change over time in athymic nude mice with MMTV-HER2 Fo5 Mammary tumor allografts dosed with 650 $\mu\text{g}/\text{m}^2$ Trastuzumab-MC-MMAF. Table 2d and FIGS. 13-15 show that the ADC have strong anti-tumor activity in the allograft of a HER2 positive tumor (Fo5) that originally arose in an MMTV-HER2 transgenic mouse. The antibody alone (e.g., Trastuzumab) does not have significant anti-tumor activity in this model (Erickson et al. U.S. Pat. No. 6,632,979). As illustrated in FIGS. 13-15, the growth of the tumors was retarded by treatment with ADC as compared to control (Vehicle) level of growth.

In a surprising and unexpected discovery, the in vivo anti-tumor activity results of the ADC in Table 2d show generally that ADC with a low average number of drug moieties per antibody showed efficacy, e.g., tumor doubling time >15 days and mean log cell kill >1.0. FIG. 16 shows that for the antibody drug conjugate, trastuzumab-MC-vc-PAB-MMAF, the mean tumor volume diminished and did not progress where the MMAF:trastuzumab ratio was 2 and 4, whereas tumor progressed at a ratio of 5.9 and 6, but at a rate lower than Vehicle (buffer). The rate of tumor progression in this mouse xenograft model was about the same, i.e. 3 days, for Vehicle and trastuzumab. The results suggest that at least for trastuzumab ADC, the optimal ratio of drug moieties per antibody may be less than about 8, and may be about 2 to about 4.

9.5.5 Rodent Toxicity

Antibody drug conjugates and an ADC-minus control, "Vehicle", were evaluated in an acute toxicity rat model. Toxicity of ADC was investigated by treatment of male and female Sprague-Dawley rats with the ADC and subsequent inspection and analysis of the effects on various organs. Gross observations included changes in body weights and signs of lesions and bleeding. Clinical pathology parameters (serum chemistry and hematology), histopathology, and necropsy were conducted on dosed animals.

It is considered that weight loss, or weight change relative to animals dosed only with Vehicle, in animals after dosing with ADC is a gross and general indicator of systemic or localized toxicity. FIGS. 17-19 show the effects of various ADC and control (Vehicle) after dosing on rat body weight.

Hepatotoxicity was measured by elevated liver enzymes, increased numbers of mitotic and apoptotic figures and hepatocyte necrosis. Hematolymphoid toxicity was

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observed by depletion of leukocytes, primarily granulocytes (neutrophils), and/or platelets, and lymphoid organ involvement, i.e. atrophy or apoptotic activity. Toxicity was also noted by gastrointestinal tract lesions such as increased numbers of mitotic and apoptotic figures and degenerative enterocolitis.

Enzymes indicative of liver injury that were studied include:

AST (aspartate aminotransferase)

Localization: cytoplasmic; liver, heart, skeletal muscle, kidney

Liver:Plasma ratio of 7000:1

T1/2: 17 hrs

ALT (alanine aminotransferase)

Localization: cytoplasmic; liver, kidney, heart, skeletal muscle

Liver:Plasma ratio of 3000:1

T1/2: 42 hrs; diurnal variation

GGT (g-glutamyl transferase)

Localization: plasma membrane of cells with high secretory or absorptive capacity; liver, kidney, intestine

Poor predictor of liver injury; commonly elevated in bile duct disorders

The toxicity profiles of trastuzumab-MC-val-cit-MMAF, trastuzumab-MC(Me)-val-cit-PAB-MMAF, trastuzumab-MC-MMAF and trastuzumab-MC-val-cit-PAB-MMAF were studied in female Sprague-Dawley rats (Example 19). The humanized trastuzumab antibody does not bind appreciably to rat tissue, and any toxicity would be considered non-specific. Variants at dose levels of 840 and 2105 $\mu\text{g}/\text{m}^2$ MMAF were compared to trastuzumab-MC-val-cit-PAB-MMAF at 2105 $\mu\text{g}/\text{m}^2$.

Animals in groups 1, 2, 3, 4, 6, and 7 (Vehicle, 9.94 & 24.90 mg/kg trastuzumab-MC-val-cit-MMAF, 10.69 mg/kg trastuzumab-MC(Me)-val-cit-PAB-MMAF, and 10.17 & 25.50 mg/kg trastuzumab-MC-MMAF, respectively) gained weight during the study. Animals in groups 5 and 8 (26.78 mg/kg trastuzumab-MC(Me)-val-cit-PAB-MMAF and 21.85 mg/kg trastuzumab-MC-val-cit-PAB-MMAF, respectively) lost weight during the study. On Study Day 5, the change in body weights of animals in groups 2, 6 and 7 were not significantly different from group 1 animals. The change in body weights of animals in groups 3, 4, 5 and 8 were statistically different from group 1 animals (Example 19).

Rats treated with trastuzumab-MC-MMAF (groups 6 and 7) were indistinguishable from vehicle-treated control animals at both dose levels; i.e. this conjugate showed a superior safety profile in this model. Rats treated with trastuzumab-MC-val-cit-MMAF (without the self-immolative PAB moiety; groups 2 and 3) showed dose-dependent changes typical for MMAF conjugates; the extent of the changes was less compared with a full length MC-val-cit-PAB-MMAF conjugate (group 8). The platelet counts on day 5 were at approximately 30% of baseline values in animals of group 3 (high dose trastuzumab-MC-val-cit-MMAF) compared with 15% in animals of group 8 (high dose trastuzumab-MC-val-cit-PAB-MMAF). Elevation of liver enzymes AST and ALT, of bilirubin and the extent of thrombocytopenia was most evident in animals treated with trastuzumab-MC(Me)-val-cit-PAB-MMAF (groups 4 and 5) in a dose-dependent fashion; animals of group 5 (high dose group) showed on day 5 levels of ALT of approximately 10x the baseline value and platelets were reduced by approximately 90% at the time of necropsy.

Female Sprague Dawley Rats were also dosed at high levels (Example 19, High Dose study: Groups 2, 3, 4) with

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trastuzumab-MC-MMAF, and Vehicle control (Group 1). Mild toxicity signals were observed, including a dose-dependent elevation of liver enzymes ALT, AST and GGT. On day 5 animals in the highest dose group showed a 2-fold elevation of ALT and a 5-fold elevation of AST; GGT is also elevated (6 U/L). Enzyme levels show a trend towards normalization on day 12. There was a mild granulocytosis in all three dose groups on day 5, the platelet count remained essentially unchanged in all animals. Morphological changes were mild; animals treated at the 4210 $\mu\text{g}/\text{m}^2$ dose level (Group 2) showed unremarkable histology of liver, spleen, thymus, intestines and bone marrow. Mildly increased apoptotic and mitotic activity was observed in thymus and liver, respectively in animals treated at the 5500 $\mu\text{g}/\text{m}^2$ dose level (Group 3). The bone marrow was normocellular, but showed evidence of granulocytic hyperplasia, which is consistent with the absolute granulocytosis observed in the peripheral blood counts in these animals. Animals at the highest dose in group 4 showed qualitatively the same features; the mitotic activity in the liver appears somewhat increased compared to animals in Group 3. Also, extramedullary hematopoiesis was seen in spleen and liver.

EphB2R is a type 1 TM tyrosine kinase receptor with close homology between mouse and human, and is over-expressed in colorectal cancer cells. 2H9 is an antibody against EphB2R. The naked antibody has no effect on tumor growth, but 2H9-val-cit-MMAE killed EphB2R expressing cells and showed efficacy in a mouse xenograft model using CFX1103 human colon tumors (Mao et al (2004) Cancer Res. 64:781-788). 2H9 and 7C₂ are both mouse IgG1 anti-HER2 antibodies. The toxicity profiles of 2H9-MC-val-cit-PAB-MMAF (3.7 MMAF/Ab), 7C₂-MC-val-cit-PAB-MMAF (4 MMAF/Ab), and trastuzumab-MC-val-cit-PAB-MMAF (5.9 MMAF/Ab) were compared. The differences in the structure of each immunoconjugate or the drug portion of the immunoconjugate may affect the pharmacokinetics and ultimately the safety profile. The humanized trastuzumab antibody does not bind appreciably to rat tissue, and any toxicity would be considered non-specific.

9.5.6 Cynomolgus Monkey Toxicity/Safety

Similar to the rat toxicity/safety study, cynomolgus monkeys were treated with ADC followed by liver enzyme measurements, and inspection and analysis of the effects on various organs. Gross observations included changes in body weights and signs of lesions and bleeding. Clinical pathology parameters (serum chemistry and hematology), histopathology, and necropsy were conducted on dosed animals (Example 19).

The antibody drug conjugate, H-MC-vc-PAB-MMAE (H=trastuzumab linked through cysteine) showed no evidence of liver toxicity at any of the dose levels tested. Peripheral blood granulocytes showed depletion after a single dose of 1100 mg/m^2 with complete recovery 14 days post-dose. The antibody drug conjugate H-MC-vc-PAB-MMAF showed elevation of liver enzymes at 550 (transient) and 880 mg/m^2 dose level, no evidence of granulocytopenia, and a dose-dependent, transient (groups 2 & 3) decline of platelets.

9.6 Synthesis of the Compounds of the Invention

The Exemplary Compounds and Exemplary Conjugates can be made using the synthetic procedures outlined below in FIGS. 25-36. As described in more detail below, the Exemplary Compounds or Exemplary Conjugates can be conveniently prepared using a Linker having a reactive site for binding to the Drug and Ligand. In one aspect, a Linker

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has a reactive site which has an electrophilic group that is reactive to a nucleophilic group present on a Ligand, such as but not limited to an antibody. Useful nucleophilic groups on an antibody include but are not limited to, sulfhydryl, hydroxyl and amino groups. The heteroatom of the nucleophilic group of an antibody is reactive to an electrophilic group on a Linker and forms a covalent bond to a Linker unit. Useful electrophilic groups include, but are not limited to, maleimide and haloacetamide groups. The electrophilic group provides a convenient site for antibody attachment.

In another embodiment, a Linker has a reactive site which has a nucleophilic group that is reactive to an electrophilic group present on an antibody. Useful electrophilic groups on an antibody include, but are not limited to, aldehyde and ketone carbonyl groups. The heteroatom of a nucleophilic group of a Linker can react with an electrophilic group on an antibody and form a covalent bond to an antibody unit. Useful nucleophilic groups on a Linker include, but are not limited to, hydrazide, oxime, amino, hydrazine, thiosemicarbazone, hydrazine carboxylate, and arylhydrazide. The electrophilic group on an antibody provides a convenient site for attachment to a Linker.

Carboxylic acid functional groups and chloroformate functional groups are also useful reactive sites for a Linker because they can react with secondary amino groups of a Drug to form an amide linkage. Also useful as a reactive site is a carbonate functional group on a Linker, such as but not limited to p-nitrophenyl carbonate, which can react with an amino group of a Drug, such as but not limited to N-methyl valine, to form a carbamate linkage. Typically, peptide-based Drugs can be prepared by forming a peptide bond between two or more amino acids and/or peptide fragments. Such peptide bonds can be prepared, for example, according to the liquid phase synthesis method (see E. Schroder and K. Lübke, "The Peptides", volume 1, pp 76-136, 1965, Academic Press) that is well known in the field of peptide chemistry.

The synthesis of an illustrative Stretcher having an electrophilic maleimide group is illustrated below in FIGS. 28 and 29. General synthetic methods useful for the synthesis of a Linker are described in FIG. 30. FIG. 31 shows the construction of a Linker unit having a val-cit group, an electrophilic maleimide group and a PAB self-immolative Spacer group. FIG. 32 depicts the synthesis of a Linker having a phe-lys group, an electrophilic maleimide group, with and without the PAB self-immolative Spacer group. FIG. 33 presents a general outline for the synthesis of a Drug-Linker Compound, while FIG. 34 presents an alternate route for preparing a Drug-Linker Compound. FIG. 35 depicts the synthesis of a branched linker containing a BHMS group. FIG. 36 outlines the attachment of an antibody to a Drug-Linker Compound to form a Drug-Linker-Antibody Conjugate, and FIG. 34 illustrates the synthesis of Drug-Linker-Antibody Conjugates having, for example but not limited to, 2 or 4 drugs per Antibody.

As described in more detail below, the Exemplary Conjugates are conveniently prepared using a Linker having two or more Reactive Sites for binding to the Drug and a Ligand. In one aspect, a Linker has a Reactive site which has an electrophilic group that is reactive to a nucleophilic group present on a Ligand, such as an antibody. Useful nucleophilic groups on an antibody include but are not limited to, sulfhydryl, hydroxyl and amino groups. The heteroatom of the nucleophilic group of an antibody is reactive to an electrophilic group on a Linker and forms a covalent bond to a Linker unit. Useful electrophilic groups include, but are

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not limited to, maleimide and haloacetamide groups. The electrophilic group provides a convenient site for antibody attachment.

In another embodiment, a Linker has a Reactive site which has a nucleophilic group that is reactive to an electrophilic group present on a Ligand, such as an antibody. Useful electrophilic groups on an antibody include, but are not limited to, aldehyde and ketone carbonyl groups. The heteroatom of a nucleophilic group of a Linker can react with an electrophilic group on an antibody and form a covalent bond to an antibody unit. Useful nucleophilic groups on a Linker include, but are not limited to, hydrazide, oxime, amino, hydrazine, thiosemicarbazone, hydrazine carboxylate, and arylhydrazide. The electrophilic group on an antibody provides a convenient site for attachment to a Linker.

9.6.1 Drug Moiety Synthesis

Typically, peptide-based Drugs can be prepared by forming a peptide bond between two or more amino acids and/or peptide fragments. Such peptide bonds can be prepared, for example, according to the liquid phase synthesis method (see E. Schröder and K. Lübke, "The Peptides", volume 1, pp 76-136, 1965, Academic Press) that is well known in the field of peptide chemistry.

The auristatin/dolastatin drug moieties may be prepared according to the general methods of: U.S. Pat. Nos. 5,635, 483; 5,780,588; Pettit et al. (1989) J. Am. Chem. Soc. 111:5463-5465; Pettit et al. (1998) Anti-Cancer Drug Design 13:243-277; and Pettit et al. (1996) J. Chem. Soc. Perkin Trans. 1 5:859-863.

In one embodiment, a Drug is prepared by combining about a stoichiometric equivalent of a dipeptide and a tripeptide, preferably in a one-pot reaction under suitable condensation conditions. This approach is illustrated in FIGS. 25-27, below.

FIG. 25 illustrates the synthesis of an N-terminal tripeptide unit F which is a useful intermediate for the synthesis of the drug compounds of Formula Ib.

As illustrated in FIG. 25, a protected amino acid A (where PG represents an amine protecting group, R⁴ is selected from hydrogen, C₁-C₈ alkyl, C₃-C₈ carbocycle, —O—(C₁-C₈ alkyl), -aryl, alkyl-aryl, alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle, alkyl-(C₃-C₈ heterocycle) wherein R⁵ is selected from H and methyl; or R⁴ and R⁵ join, have the formula —(CR^aR^b)_n— wherein R^a and R^b are independently selected from hydrogen, C₁-C₈ alkyl and C₃-C₈ carbocycle and n is selected from 2, 3, 4, 5 and 6, and form a ring with the carbon atom to which they are attached) is coupled to t-butyl ester B (where R⁶ is selected from —H and —C₁-C₈ alkyl; and IC is selected from hydrogen, C₁-C₈ alkyl, C₃-C₈ carbocycle, —O—(C₁-C₈ alkyl), -aryl, alkyl-aryl, alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and alkyl-(C₃-C₈ heterocycle)) under suitable coupling conditions, e.g., in the presence of PyBrop and diisopropylethylamine, or using DCC (see, for example, Miyazaki, K. et. al. *Chem. Pharm. Bull.* 1995, 43(10), 1706-1718).

Suitable protecting groups PG, and suitable synthetic methods to protect an amino group with a protecting group are well known in the art. See, e.g., Greene, T. W. and Wuts, P. G. M., *Protective Groups in Organic Synthesis*, 2nd Edition, 1991, John Wiley & Sons. Exemplary protected amino acids A are PG-Ile and, particularly, PG-Val, while other suitable protected amino acids include, without limitation: PG-cyclohexylglycine, PG-cyclohexylalanine, PG-aminocyclopropane-1-carboxylic acid, PG-aminoisobutyric acid, PG-phenylalanine, PG-phenylglycine, and PG-tert-butylglycine. Z is an exemplary protecting group. Fmoc

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is another exemplary protecting group. An exemplary t-butyl ester B is dolaisoleuine t-butyl ester.

The dipeptide C can be purified, e.g., using chromatography, and subsequently deprotected, e.g., using H₂ and 10% Pd—C in ethanol when PG is benzyloxycarbonyl, or using diethylamine for removal of an Fmoc protecting group. The resulting amine D readily forms a peptide bond with an amino acid BB (wherein R¹ is selected from —H, —C₁-C₈ alkyl and —C₃-C₈ carbocycle; and R² is selected from —H and —C₁-C₈ alkyl; or R¹ and R² join, have the formula —(CR^aR^b)_n— wherein R^a and R^b are independently selected from —H, —C₁-C₈ alkyl and —C₃-C₈ carbocycle and n is selected from 2, 3, 4, 5 and 6, and form a ring with the nitrogen atom to which they are attached; and R³ is selected from hydrogen, C₁-C₈ alkyl, C₃-C₈ carbocycle, —O—(C₁-C₈ alkyl), -aryl, alkyl-aryl, alkyl-(C₃-C₈ carbocycle), C₃-C₈ heterocycle and alkyl-(C₃-C₈ heterocycle)). N,N-Dialkyl amino acids are exemplary amino acids for BB, such as commercially available N,N-dimethyl valine. Other N,N-dialkyl amino acids can be prepared by reductive bis-alkylation using known procedures (see, e.g., Bowman, R. E., Stroud, H. H. *J. Chem. Soc.*, 1950, 1342-1340). Fmoc-Me-L-Val and Fmoc-Me-L-glycine are two exemplary amino acids BB useful for the synthesis of N-mono-alkyl derivatives. The amine D and the amino acid BB react to provide the tripeptide E using coupling reagent DEPC with triethylamine as the base. The C-terminus protecting group of E is subsequently deprotected using HCl to provide the tripeptide compound of formula F.

Illustrative DEPC coupling methodology and the PyBrop coupling methodology shown in FIG. 25 are outlined below in General Procedure A and General Procedure B, respectively. Illustrative methodology for the deprotection of a Z-protected amine via catalytic hydrogenation is outlined below in General Procedure C.

General Procedure A: Peptide Synthesis Using DEPC.

The N-protected or N, N-disubstituted amino acid or peptide D (1.0 eq.) and an amine BB (1.1 eq.) are diluted with an aprotic organic solvent, such as dichloromethane (0.1 to 0.5 M). An organic base such as triethylamine or diisopropylethylamine (1.5 eq.) is then added, followed by DEPC (1.1 eq.). The resulting solution is stirred, preferably under argon, for up to 12 hours while being monitored by HPLC or TLC. The solvent is removed in vacuo at room temperature, and the crude product is purified using, for example, HPLC or flash column chromatography (silica gel column). Relevant fractions are combined and concentrated in vacuo to afford tripeptide E which is dried under vacuum overnight.

General Procedure B: Peptide Synthesis Using PyBrop.

The amino acid B (1.0 eq.), optionally having a carboxyl protecting group, is diluted with an aprotic organic solvent such as dichloromethane or DME to provide a solution of a concentration between 0.5 and 1.0 mM, then diisopropylethylamine (1.5 eq.) is added. Fmoc-, or Z-protected amino acid A (1.1 eq.) is added as a solid in one portion, then PyBrop (1.2 eq.) is added to the resulting mixture. The reaction is monitored by TLC or HPLC, followed by a workup procedure similar to that described in General Procedure A.

General Procedure C: Z-Removal Via Catalytic Hydrogenation.

Z-protected amino acid or peptide C is diluted with ethanol to provide a solution of a concentration between 0.5 and 1.0 mM in a suitable vessel, such as a thick-walled round bottom flask. 10% palladium on carbon is added (5-10% w/w) and the reaction mixture is placed under a

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hydrogen atmosphere. Reaction progress is monitored using HPLC and is generally complete within 1-2 h. The reaction mixture is filtered through a pre-washed pad of celite and the celite is again washed with a polar organic solvent, such as methanol after filtration. The eluent solution is concentrated in vacuo to afford a residue which is diluted with an organic solvent, preferably toluene. The organic solvent is then removed in vacuo to afford the deprotected amine C.

FIG. 26 shows a method useful for making a C-terminal dipeptide of formula K and a method for coupling the dipeptide of formula K with the tripeptide of formula F to make drug compounds of Formula Ib.

The dipeptide K can be readily prepared by condensation of the modified amino acid Boc-Dolaproine G (see, for example, Pettit, G. R., et al. *Synthesis*, 1996, 719-725), with an amine of formula H using condensing agents well known for peptide chemistry, such as, for example, DEPC in the presence of triethylamine, as shown in FIG. 25.

The dipeptide of formula K can then be coupled with a tripeptide of formula F using General Procedure D to make the Fmoc-protected drug compounds of formula L which can be subsequently deprotected using General Procedure E in order to provide the drug compounds of formula (Ib).

General procedure D: Drug Synthesis.

A mixture of dipeptide K (1.0 eq.) and tripeptide F (1 eq.) is diluted with an aprotic organic solvent, such as dichloromethane, to form a 0.1M solution, then a strong acid, such as trifluoroacetic acid (1/2 v/v) is added and the resulting mixture is stirred under a nitrogen atmosphere for two hours at 0° C. The reaction can be monitored using TLC or, preferably, HPLC. The solvent is removed in vacuo and the resulting residue is azeotropically dried twice, preferably using toluene. The resulting residue is dried under high vacuum for 12 h and then diluted with an aprotic organic solvent, such as dichloromethane. An organic base such as triethylamine or diisopropylethylamine (1.5 eq.) is then added, followed by either PyBrop (1.2 eq.) or DEPC (1.2 eq.) depending on the chemical functionality on the residue. The reaction mixture is monitored by either TLC or HPLC and upon completion, the reaction is subjected to a workup procedure similar or identical to that described in General Procedure A.

General Procedure E: Fmoc-Removal Using Diethylamine.

An Fmoc-protected Drug L is diluted with an aprotic organic solvent such as dichloromethane and to the resulting solution is added diethylamine (1/2 v/v). Reaction progress is monitored by TLC or HPLC and is typically complete within 2 h. The reaction mixture is concentrated in vacuo and the resulting residue is azeotropically dried, preferably using toluene, then dried under high vacuum to afford Drug Ib having a deprotected amino group.

FIG. 27 shows a method useful for making MMAF derivatives of Formula (Ib).

The dipeptide O can be readily prepared by condensation of the modified amino acid Boc-Dolaproine G (see, for example, Pettit, G. R., et al. *Synthesis*, 1996, 719-725), with a protected amino acid of formula III using condensing agents well known for peptide chemistry, such as, for example, DEPC in the presence of triethylamine, as shown in FIGS. 25 and 26.

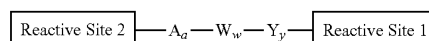
The dipeptide of formula O can then be coupled with a tripeptide of formula F using General Procedure D to make the Fmoc-protected MMAF compounds of formula P which can be subsequently deprotected using General Procedure E in order to provide the MMAF drug compounds of formula (Ib).

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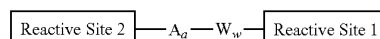
Thus, the above methods are useful for making Drugs that can be used in the present invention.

9.6.2 Drug Linker Synthesis

To prepare a Drug-Linker Compound of the present invention, the Drug is reacted with a reactive site on the Linker. In general, the Linker can have the structure:

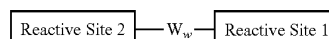


when both a Spacer unit (—Y—) and a Stretcher unit (—A—) are present. Alternately, the Linker can have the structure:



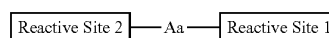
when the Spacer unit (—Y—) is absent.

The Linker can also have the structure:



when both the Stretcher unit (—A—) and the Spacer unit (—Y—) are absent.

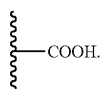
The Linker can also have the structure:



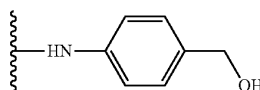
when both the Amino Acid unit (W) and the Spacer Unit (Y) are absent.

In general, a suitable Linker has an Amino Acid unit linked to an optional Stretcher Unit and an optional Spacer Unit. Reactive Site 1 is present at the terminus of the Spacer and Reactive site 2 is present at the terminus of the Stretcher. If a Spacer unit is not present, then Reactive site 1 is present at the C-terminus of the Amino Acid unit.

In an exemplary embodiment of the invention, Reactive Site No. 1 is reactive to a nitrogen atom of the Drug, and Reactive Site No. 2 is reactive to a sulfhydryl group on the Ligand. Reactive Sites 1 and 2 can be reactive to different functional groups.



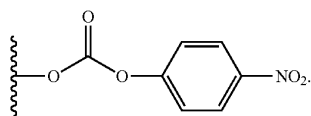
In one aspect of the invention, Reactive Site No. 1 is
In another aspect of the invention, Reactive Site No. 1 is



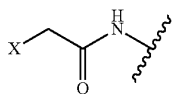
In still another aspect of the invention, Reactive Site No. 1 is a p-nitrophenyl carbonate having the formula

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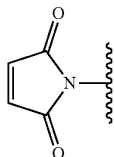
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In one aspect of the invention, Reactive Site No. 2 is a thiol-accepting group. Suitable thiol-accepting groups include haloacetamide groups having the formula



wherein X represents a leaving group, preferably O-methyl, O-tosyl, —Cl, —Br, or —I; or a maleimide group having the formula



Useful Linkers can be obtained via commercial sources, such as Molecular Biosciences Inc. (Boulder, Colo.), or prepared as summarized in FIGS. 28-30.

In FIG. 28 X is —CH₂— or —CH₂OCH₂—; and n is an integer ranging either from 0-10 when X is —CH₂—; or 1-10 when X is —CH₂OCH₂—.

The method shown in FIG. 29 combines maleimide with a glycol under Mitsunobu conditions to make a polyethylene glycol maleimide Stretcher (see for example, Walker, M. A. *J. Org. Chem.* 1995, 60, 5352-5), followed by installation of a p-nitrophenyl carbonate Reactive Site group.

In FIG. 29 E is —CH₂— or —CH₂OCH₂—; and e is an integer ranging from 0-8;

Alternatively, PEG-maleimide and PEG-haloacetamide stretchers can be prepared as described by Frisch, et al., *Bioconjugate Chem.* 1996, 7, 180-186.

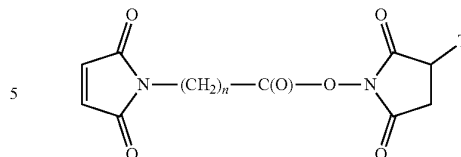
FIG. 30 illustrates a general synthesis of an illustrative Linker unit containing a maleimide Stretcher group and optionally a p-aminobenzyl ether self-immolative Spacer.

In FIG. 30 Q is —C₁-C₈ alkyl, —O—(C₁-C₈ alkyl), -halogen, -nitro or -cyano; m is an integer ranging from 0-4; and n is an integer ranging from 0-10.

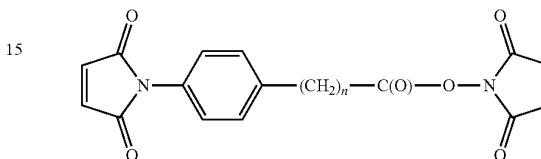
Useful Stretchers may be incorporated into a Linker using the commercially available intermediates from Molecular Biosciences (Boulder, Colo.) described below by utilizing known techniques of organic synthesis.

Stretchers of formula (Ma) can be introduced into a Linker by reacting the following intermediates with the N-terminus of an Amino Acid unit as depicted in FIGS. 31 and 32:

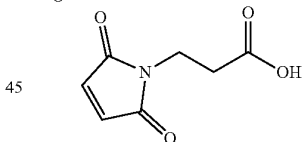
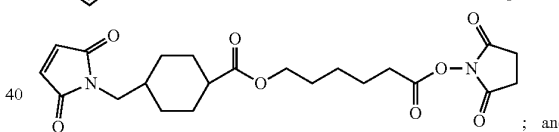
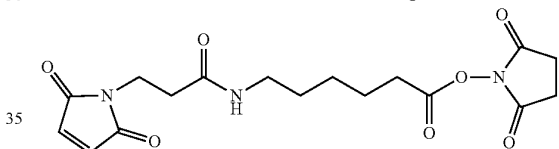
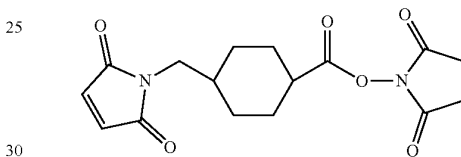
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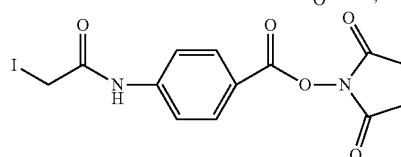
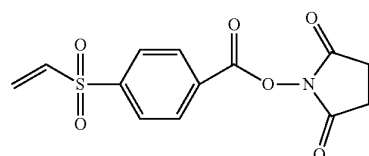
where n is an integer ranging from 1-10 and T is —H or —SO₃Na;



where n is an integer ranging from 0-3;

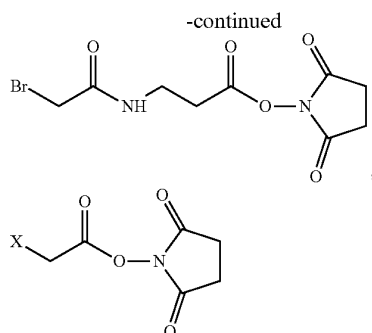


Stretcher units of formula (IIIb) can be introduced into a Linker by reacting the following intermediates with the N-terminus of an Amino Acid unit:

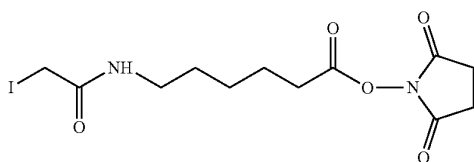


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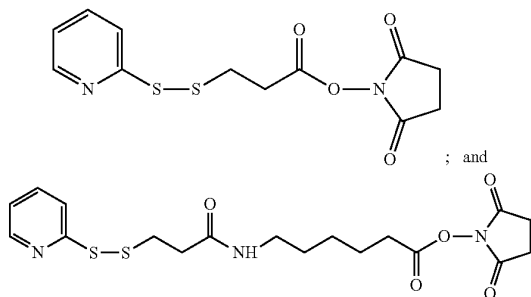
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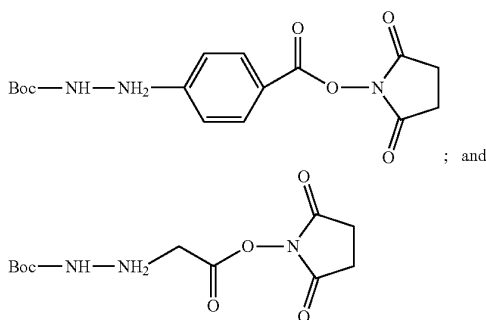
where X is —Br or —I; and



Stretcher units of formula (IV) can be introduced into a Linker by reacting the following intermediates with the N-terminus of an Amino Acid unit:



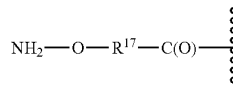
Stretcher units of formula (Va) can be introduced into a Linker by reacting the following intermediates with the N-terminus of an Amino Acid unit:



Other useful Stretches may be synthesized according to known procedures. Aminoxy Stretches of the formula shown below can be prepared by treating alkyl halides with

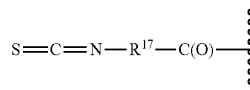
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N-Boc-hydroxylamine according to procedures described in Jones, D. S. et al., *Tetrahedron Letters*, 2000, 41(10), 1531-1533; and Gilon, C. et al., *Tetrahedron*, 1967, 23(11), 4441-4447.



wherein —R¹⁷— is selected from —C₁-C₁₀ alkylene-, —C₃-C₈ carbocyclo-, —O—(C₁-C₈ alkyl)-, -arylene-, —C₁-C₁₀ alkylene-arylene-, -arylene-C₁-C₁₀ alkylene-, alkylene-(C₃-C₈ carbocyclo)-, —(C₃-C₈ carbocyclo)-C₁-C₁₀ alkylene-, —C₃-C₈ heterocyclo-, —C₁-C₁₀ alkylene-(C₃-C₈ heterocyclo)-, —(C₃-C₈ heterocyclo)-C₁-C₁₀ alkylene-, —(CH₂CH₂O)_r—, —(CH₂CH₂O)_r—CH₂—; and r is an integer ranging from 1-10;

Isothiocyanate Stretches of the formula shown below may be prepared from isothiocyanatocarboxylic acid chlorides as described in *Angew. Chem.*, 1975, 87(14):517.



wherein —R¹⁷— is as described herein.

FIG. 31 shows a method for obtaining of a val-cit dipeptide Linker having a maleimide Stretcher and optionally a p-aminobenzyl self-immolative Spacer.

In FIG. 31 Q is —C₁-C₈ alkyl, —O—(C₁-C₈ alkyl), -halogen, -nitro or -cyano; and m is an integer ranging from 0-4.

FIG. 32 illustrates the synthesis of a phe-lys(Mtr) dipeptide Linker unit having a maleimide Stretcher unit and a p-aminobenzyl self-immolative Spacer unit. Starting material AD (lys(Mtr)) is commercially available (Bachem, Torrance, Calif.) or can be prepared according to Dubowchik, et al. *Tetrahedron Letters* (1997) 38:5257-60.

In FIG. 32 Q is —C₁-C₈ alkyl, —O—(C₁-C₈ alkyl), -halogen, -nitro or -cyano; and m is an integer ranging from 0-4.

As shown in FIG. 33, a Linker can be reacted with an amino group of a Drug Compound of Formula (Ib) to form a Drug-Linker Compound that contains an amide or carbamate group, linking the Drug unit to the Linker unit. When Reactive Site No. 1 is a carboxylic acid group, as in Linker AJ, the coupling reaction can be performed using HATU or PyBrop and an appropriate amine base, resulting in a Drug-Linker Compound AK, containing an amide bond between the Drug unit and the Linker unit. When Reactive Site No. 1 is a carbonate, as in Linker AL, the Linker can be coupled to the Drug using HOBt in a mixture of DMF/pyridine to provide a Drug-Linker Compound AM, containing a carbamate bond between the Drug unit and the Linker unit.

Alternatively, when Reactive Site No. 1 is a good leaving group, such as in Linker AN, the Linker can be coupled with an amine group of a Drug via a nucleophilic substitution process to provide a Drug-Linker Compound having an amine linkage (AO) between the Drug unit and the Linker unit.

Illustrative methods useful for linking a Drug to a Ligand to form a Drug-Linker Compound are depicted in FIG. 33 and are outlined in General Procedures G-H.

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General Procedure G: Amide Formation Using HATU.

A Drug (Ib) (1.0 eq.) and an N-protected Linker containing a carboxylic acid Reactive site (1.0 eq.) are diluted with a suitable organic solvent, such as dichloromethane, and the resulting solution is treated with HATU (1.5 eq.) and an organic base, preferably pyridine (1.5 eq.). The reaction mixture is allowed to stir under an inert atmosphere, preferably argon, for 6 h, during which time the reaction mixture is monitored using HPLC. The reaction mixture is concentrated and the resulting residue is purified using HPLC to yield the amide of formula AK.

Procedure H: Carbamate Formation Using HOBT.

A mixture of a Linker AL having a p-nitrophenyl carbonate Reactive site (1.1 eq.) and Drug (Ib) (1.0 eq.) are diluted with an aprotic organic solvent, such as DMF, to provide a solution having a concentration of 50-100 mM, and the resulting solution is treated with HOBt (2.0 eq.) and placed under an inert atmosphere, preferably argon. The reaction mixture is allowed to stir for 15 min, then an organic base, such as pyridine (1/4 v/v), is added and the reaction progress is monitored using HPLC. The Linker is typically consumed within 16 h. The reaction mixture is then concentrated in vacuo and the resulting residue is purified using, for example, HPLC to yield the carbamate AM.

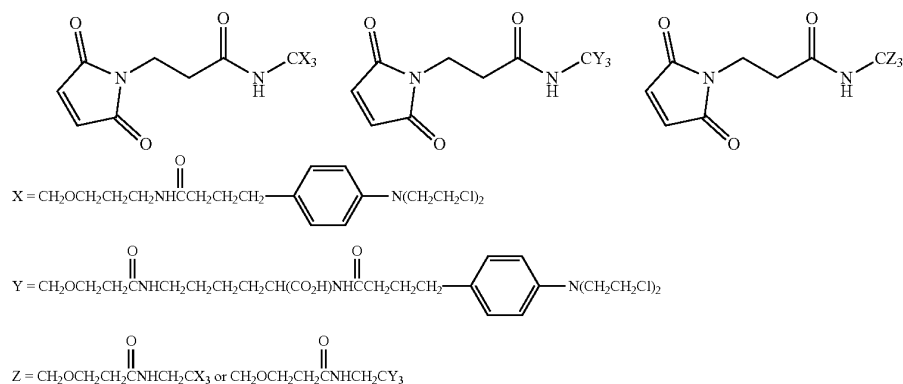
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G.; Vanelle, P.; Nouguié, R. *Tetrahedron Lett.* (1985) 26:5133-5134) and utilizes as starting materials, commercially available diethyl (4-nitrobenzyl)phosphonate (AT) and commercially available 2,2-dimethyl-1,3-dioxan-5-one (AU). Linkers AY and BA can be prepared from intermediate AW using the methodology described in FIG. 29.

9.6.3 Dendritic Linkers

The linker may be a dendritic type linker for covalent attachment of more than one drug moiety through a branching, multifunctional linker moiety to a Ligand, such as but not limited to an antibody (Sun et al. (2002) *Bioorganic & Medicinal Chemistry Letters* 12:2213-2215; Sun et al. (2003) *Bioorganic & Medicinal Chemistry* 11:1761-1768). Dendritic linkers can increase the molar ratio of drug to antibody, i.e. loading, which is related to the potency of the Drug-Linker-Ligand Conjugate. Thus, where a cysteine engineered antibody bears only one reactive cysteine thiol group, a multitude of drug moieties may be attached through a dendritic linker.

The following exemplary embodiments of dendritic linker reagents allow up to nine nucleophilic drug moiety reagents to be conjugated by reaction with the chloroethyl nitrogen mustard functional groups:



An alternate method of preparing Drug-Linker Compounds is outlined in FIG. 34. Using the method of FIG. 34, the Drug is attached to a partial Linker unit (ZA, for example), which does not have a Stretcher unit attached. This provides intermediate AP, which has an Amino Acid unit having an Fmoc-protected N-terminus. The Fmoc group is then removed and the resulting amine intermediate AQ is then attached to a Stretcher unit via a coupling reaction catalyzed using PyBrop or DEPC. The construction of Drug-Linker Compounds containing either a bromoacetamide Stretcher AR or a PEG maleimide Stretcher AS is illustrated in FIG. 34.

In FIG. 34 Q is $-\text{C}_1\text{-C}_8$ alkyl, $-\text{O}-(\text{C}_1\text{-C}_8 \text{ alkyl})$, -halogen, -nitro or -cyano; and m is an integer ranging from 0-4.

Methodology useful for the preparation of a Linker unit 60 containing a branched spacer is shown in FIG. 35.

FIG. 35 illustrates the synthesis of a val-cit dipeptide linker having a maleimide Stretcher unit and a bis(4-hydroxymethyl)styrene (BHMS) unit. The synthesis of the BHMS intermediate (AW) has been improved from previous literature procedures (see International Publication No. WO 9813059 to Firestone et al., and Crozet, M. P.; Archaimbault,

9.6.4 Conjugation of Drug Moieties to Antibodies

FIG. 36 illustrates methodology useful for making Drug-Linker-Ligand conjugates having about 2 to about 4 drugs per antibody. An antibody is treated with a reducing agent, such as dithiothreitol (DTT) to reduce some or all of the cysteine disulfide residues to form highly nucleophilic cysteine thiol groups ($-\text{CH}_2\text{SH}$). The partially reduced antibody thus reacts with drug-linker compounds, or linker reagents, with electrophilic functional groups such as maleimide or α -halo carbonyl, according to the conjugation method at page 766 of Klussman, et al. (2004), *Bioconjugate Chemistry* 15(4):765-773.

For example, an antibody, e.g., AC10, dissolved in 500 mM sodium borate and 500 mM sodium chloride at pH 8.0 is treated with an excess of 100 mM dithiothreitol (DTT). After incubation at 37° C. for about 30 minutes, the buffer is exchanged by elution over Sephadex G25 resin and eluted with PBS with 1 mM DTPA. The thiol/Ab value is checked by determining the reduced antibody concentration from the absorbance at 280 nm of the solution and the thiol concentration by reaction with DTNB (Aldrich, Milwaukee, Wis.) and determination of the absorbance at 412 nm. The reduced antibody dissolved in PBS is chilled on ice. The drug linker,

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e.g., MC-val-cit-PAB-MMAE in DMSO, dissolved in acetonitrile and water at known concentration, is added to the chilled reduced antibody in PBS. After about one hour, an excess of maleimide is added to quench the reaction and cap any unreacted antibody thiol groups. The reaction mixture is concentrated by centrifugal ultrafiltration and the ADC, e.g., AC10-MC-vc-PAB-MMAE, is purified and desalted by elution through G25 resin in PBS, filtered through 0.2 μ m filters under sterile conditions, and frozen for storage.

A variety of antibody drug conjugates (ADC) were prepared, with a variety of linkers, and the drug moieties, MMAE and MMAF. The following table is an exemplary group of ADC which were prepared following the protocol of Example 27, and characterized by HPLC and drug loading assay.

Target (antigen)	ADC	isolated amount (mg)	drug/ Ab ratio
0772P	16E12-MC-vc-PAB-MMAE	1.75	
0772P	11D10-MC-vc-PAB-MMAE	46.8	.4
0772P	11D10-MC-vc-PAB-MMAF	54.5	.8
Brevican	Brevican-MC-MMAF	2	
Brevican	Brevican-MC-vc-MMAF	2	
Brevican	Brevican-MC-vc-PAB-MMAF	1.4	
CD21	CD21-MC-vc-PAB-MMAE	38.1	.3
CD21	CD21-MC-vc-PAB-MMAF	43	.1
CRIPTO	11F4-MC-vc-PAB-MMAF	6	.8
CRIPTO	25G8-MC-vc-PAB-MMAF	7.4	.7
E16	12G12-MC-vc-PAB-MMAE	2.3	.6
E16	3B5-MC-vc-PAB-MMAE	2.9	.6
E16	12B9-MC-vc-PAB-MMAE	1.4	.8
E16	12B9-MC-vc-PAB-MMAE	5.1	
E16	12G12-MC-vc-PAB-MMAE	3	.6
E16	3B5-MC-vc-PAB-MMAE	4.8	.1
E16	3B5-MC-vc-PAB-MMAF	24.7	.4
EphB2R	2H9-MC-vc-PAB-MMAE	29.9	.1
EphB2R	2H9-MC-fk-PAB-MMAE	25	.5
EphB2R	2H9-MC-vc-PAB-MMAE	175	.1
EphB2R	2H9-MC-vc-PAB-MMAF	150	.8
EphB2R	2H9-MC-vc-PAB-MMAF	120	.7
EphB2R	2H9-MC-vc-PAB-MMAE	10.7	.4
IL-20Ra	IL20Ra-vc-MMAE	26	.7
IL-20Ra	IL20Ra-vc-MMAE	27	.3
ePhB2	IL8-MC-vc-PAB-MMAE	251	.7
MDP	MDP-vc-MMAE	32	
MPF	19C3-vc-MMAE	1.44	.5
MPF	7D9-vc-MMAE	4.3	.8
MPF	19C3-vc-MMAE	7.9	
MPF	7D9-MC-vc-PAB-MMAF	5	.3
Napi3b	10H1-vc-MMAE	4.5	.6
Napi3b	4C9-vc-MMAE	3.0	.4
Napi3b	10H1-vc-MMAE	4.5	.8
Napi3b	10H1-vc-MMAE	6.5	
NCA	3E6-MC-fk-PAB-MMAE	49.6	.4
NCA	3E6-MC-vc-PAB-MMAE	56.2	.4
PSCA	PSCA-fk-MMAE	51.7	.9
PSCA	PSCA-vc-MMAE	61.1	.6
Napi3b	10H1-MC-vc-PAB-MMAE	75	.2
Napi3b	10H1-MC-vc-PAB-MMAF	95	.4
Napi3b	10H1-MC-MMAF	92	
EphB2R	2H9-MC-vc-PAB-MMAE	79	
EphB2R	2H9-MC-MMAF	92	.9
0772P	11D10(Fc chimera)-MC-vc-PAB-MMAE	79	.3
0772P	11D10(Fc chimera)-MC-vc-PAB-MMAF	70	.5
0772P	11D10(Fc chimera)-MC-MMAF	23	.5
Brevican	6D2-MC-vc-PAB-MMAF	0.3	.3
Brevican	6D2-MC-MMAF	0.36	.5
EphB2R	2H9(Fc chimera)-MC-vc-PAB-MMAE	1983	.3
E16	12B9-MC-vc-PAB-MMAE	14.1	.6
E16	12B9-MC-vc-PAB-MMAF	16.4	.5
E16	12G12-MC-vc-PAB-MMAE	10.5	.1
E16	12G12-MC-vc-PAB-MMAF	10.2	.8
E16	3B5-MC-vc-PAB-MMAE	58.6	.8

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-continued

Target (antigen)	ADC	isolated amount (mg)	drug/ Ab ratio
E16	3B5-MC-vc-PAB-MMAF	8	.1
0772P	11D10(Fc chimera)-MC-vc-PAB-MMAE	340	.9
Steap1	(Steap1-92)-MC-vc-PAB-MMAE	3.5	
Steap1	(Steap1-92)-MC-vc-PAB-MMAF	4.7	
10 Steap1	(Steap1-120)-MC-vc-PAB-MMAE	2	
Steap1	(Steap1-120)-MC-vc-PAB-MMAF	2.3	
E16	3B5-MC-vc-PAB-MMAF	52.2	.5

9.7 Compositions and Methods of Administration

In other embodiments, described is a composition including an effective amount of an Exemplary Compound and/or Exemplary Conjugate and a pharmaceutically acceptable carrier or vehicle. For convenience, the Drug units and Drug-Linker Compounds can be referred to as Exemplary Compounds, while Drug-Ligand Conjugates and Drug-Linker-Ligand Conjugates can be referred to as Exemplary Conjugates. The compositions are suitable for veterinary or human administration.

The present compositions can be in any form that allows for the composition to be administered to a patient. For example, the composition can be in the form of a solid, liquid or gas (aerosol). Typical routes of administration include, without limitation, oral, topical, parenteral, sublingual, rectal, vaginal, ocular, intra-tumor, and intranasal. Parenteral administration includes subcutaneous injections, intravenous, intramuscular, intrasternal injection or infusion techniques. In one aspect, the compositions are administered parenterally. In yet another aspect, the Exemplary Compounds and/or the Exemplary Conjugates or compositions are administered intravenously.

Pharmaceutical compositions can be formulated so as to allow an Exemplary Compound and/or Exemplary Conjugate to be bioavailable upon administration of the composition to a patient. Compositions can take the form of one or more dosage units, where for example, a tablet can be a single dosage unit, and a container of an Exemplary Compound and/or Exemplary Conjugate in aerosol form can hold a plurality of dosage units.

Materials used in preparing the pharmaceutical compositions can be non-toxic in the amounts used. It will be evident to those of ordinary skill in the art that the optimal dosage of the active ingredient(s) in the pharmaceutical composition will depend on a variety of factors. Relevant factors include, without limitation, the type of animal (e.g., human), the particular form of the Exemplary Compound or Exemplary Conjugate, the manner of administration, and the composition employed.

The pharmaceutically acceptable carrier or vehicle can be particulate, so that the compositions are, for example, in tablet or powder form. The carrier(s) can be liquid, with the compositions being, for example, an oral syrup or injectable liquid. In addition, the carrier(s) can be gaseous or particulate, so as to provide an aerosol composition useful in, e.g., inhalatory administration.

When intended for oral administration, the composition is preferably in solid or liquid form, where semi-solid, semi-liquid, suspension and gel forms are included within the forms considered herein as either solid or liquid.

As a solid composition for oral administration, the composition can be formulated into a powder, granule, com-

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pressed tablet, pill, capsule, chewing gum, wafer or the like form. Such a solid composition typically contains one or more inert diluents. In addition, one or more of the following can be present: binders such as carboxymethylcellulose, ethyl cellulose, microcrystalline cellulose, or gelatin; excipients such as starch, lactose or dextrans, disintegrating agents such as alginic acid, sodium alginate, Primogel, corn starch and the like; lubricants such as magnesium stearate or Sterotex; glidants such as colloidal silicon dioxide; sweetening agents such as sucrose or saccharin, a flavoring agent such as peppermint, methyl salicylate or orange flavoring, and a coloring agent.

When the composition is in the form of a capsule, e.g., a gelatin capsule, it can contain, in addition to materials of the above type, a liquid carrier such as polyethylene glycol, cyclodextrin or a fatty oil.

The composition can be in the form of a liquid, e.g., an elixir, syrup, solution, emulsion or suspension. The liquid can be useful for oral administration or for delivery by injection. When intended for oral administration, a composition can comprise one or more of a sweetening agent, preservatives, dye/colorant and flavor enhancer. In a composition for administration by injection, one or more of a surfactant, preservative, wetting agent, dispersing agent, suspending agent, buffer, stabilizer and isotonic agent can also be included.

The liquid compositions, whether they are solutions, suspensions or other like form, can also include one or more of the following: sterile diluents such as water for injection, saline solution, preferably physiological saline, Ringer's solution, isotonic sodium chloride, fixed oils such as synthetic mono or diglycerides which can serve as the solvent or suspending medium, polyethylene glycols, glycerin, cyclodextrin, propylene glycol or other solvents; antibacterial agents such as benzyl alcohol or methyl paraben; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. A parenteral composition can be enclosed in ampoule, a disposable syringe or a multiple-dose vial made of glass, plastic or other material. Physiological saline is an exemplary adjuvant. An injectable composition is preferably sterile.

The amount of the Exemplary Compound and/or Exemplary Conjugate that is effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. In addition, in vitro or in vivo assays can optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the compositions will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances.

The compositions comprise an effective amount of an Exemplary Compound and/or Exemplary Conjugate such that a suitable dosage will be obtained. Typically, this amount is at least about 0.01% of an Exemplary Compound and/or Exemplary Conjugate by weight of the composition. When intended for oral administration, this amount can be varied to range from about 0.1% to about 80% by weight of the composition. In one aspect, oral compositions can comprise from about 4% to about 50% of the Exemplary Compound and/or Exemplary Conjugate by weight of the composition. In yet another aspect, present compositions are

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prepared so that a parenteral dosage unit contains from about 0.01% to about 2% by weight of the Exemplary Compound and/or Exemplary Conjugate.

For intravenous administration, the composition can comprise from about 0.01 to about 100 mg of an Exemplary Compound and/or Exemplary Conjugate per kg of the animal's body weight. In one aspect, the composition can include from about 1 to about 100 mg of an Exemplary Compound and/or Exemplary Conjugate per kg of the animal's body weight. In another aspect, the amount administered will be in the range from about 0.1 to about 25 mg/kg of body weight of the Exemplary Compound and/or Exemplary Conjugate.

Generally, the dosage of an Exemplary Compound and/or Exemplary Conjugate administered to a patient is typically about 0.01 mg/kg to about 2000 mg/kg of the animal's body weight. In one aspect, the dosage administered to a patient is between about 0.01 mg/kg to about 10 mg/kg of the animal's body weight, in another aspect, the dosage administered to a patient is between about 0.1 mg/kg and about 250 mg/kg of the animal's body weight, in yet another aspect, the dosage administered to a patient is between about 0.1 mg/kg and about 20 mg/kg of the animal's body weight, in yet another aspect the dosage administered is between about 0.1 mg/kg to about 10 mg/kg of the animal's body weight, and in yet another aspect, the dosage administered is between about 1 mg/kg to about 10 mg/kg of the animal's body weight.

The Exemplary Compounds and/or Exemplary Conjugate or compositions can be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.). Administration can be systemic or local. Various delivery systems are known, e.g., encapsulation in liposomes, microparticles, microcapsules, capsules, etc., and can be used to administer an Exemplary Compound and/or Exemplary Conjugate or composition. In certain embodiments, more than one Exemplary Compound and/or Exemplary Conjugate or composition is administered to a patient.

In specific embodiments, it can be desirable to administer one or more Exemplary Compounds and/or Exemplary Conjugate or compositions locally to the area in need of treatment. This can be achieved, for example, and not by way of limitation, by local infusion during surgery; topical application, e.g., in conjunction with a wound dressing after surgery; by injection; by means of a catheter; by means of a suppository; or by means of an implant, the implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. In one embodiment, administration can be by direct injection at the site (or former site) of a cancer, tumor or neoplastic or pre-neoplastic tissue. In another embodiment, administration can be by direct injection at the site (or former site) of a manifestation of an autoimmune disease.

In certain embodiments, it can be desirable to introduce one or more Exemplary Compounds and/or Exemplary Conjugate or compositions into the central nervous system by any suitable route, including intraventricular and intrathecal injection. Intraventricular injection can be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir.

Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent, or via perfusion in a fluorocarbon or synthetic pulmonary surfactant.

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In yet another embodiment, the Exemplary Compounds and/or Exemplary Conjugate or compositions can be delivered in a controlled release system, such as but not limited to, a pump or various polymeric materials can be used. In yet another embodiment, a controlled-release system can be placed in proximity of the target of the Exemplary Compounds and/or Exemplary Conjugate or compositions, e.g., the brain, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in *Medical Applications of Controlled Release*, supra, vol. 2, pp. 115-138 (1984)). Other controlled-release systems discussed in the review by Langer (*Science* 249:1527-1533 (1990)) can be used.

The term "carrier" refers to a diluent, adjuvant or excipient, with which an Exemplary

Compound and/or Exemplary Conjugate is administered. Such pharmaceutical carriers can be liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. The carriers can be saline, gum acacia, gelatin, starch paste, talc, keratin, colloidal silica, urea, and the like. In addition, auxiliary, stabilizing, thickening, lubricating and coloring agents can be used. In one embodiment, when administered to a patient, the Exemplary Compound and/or Exemplary Conjugate or compositions and pharmaceutically acceptable carriers are sterile. Water is an exemplary carrier when the Exemplary Compounds and/or Exemplary Conjugates are administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical carriers also include excipients such as starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The present compositions, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents.

The present compositions can take the form of solutions, suspensions, emulsion, tablets, pills, pellets, capsules, capsules containing liquids, powders, sustained-release formulations, suppositories, emulsions, aerosols, sprays, suspensions, or any other form suitable for use. Other examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E. W. Martin.

In an embodiment, the Exemplary Compounds and/or Exemplary Conjugates are formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to animals, particularly human beings. Typically, the carriers or vehicles for intravenous administration are sterile isotonic aqueous buffer solutions. Where necessary, the compositions can also include a solubilizing agent. Compositions for intravenous administration can optionally comprise a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where an Exemplary Compound and/or Exemplary Conjugate is to be administered by infusion, it can be dispensed, for example, with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the Exemplary Compound and/or Exemplary Conjugate is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients can be mixed prior to administration.

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Compositions for oral delivery can be in the form of tablets, lozenges, aqueous or oily suspensions, granules, powders, emulsions, capsules, syrups, or elixirs, for example. Orally administered compositions can contain one or more optionally agents, for example, sweetening agents such as fructose, aspartame or saccharin; flavoring agents such as peppermint, oil of wintergreen, or cherry; coloring agents; and preserving agents, to provide a pharmaceutically palatable preparation. Moreover, where in tablet or pill form, the compositions can be coated to delay disintegration and absorption in the gastrointestinal tract thereby providing a sustained action over an extended period of time. Selectively permeable membranes surrounding an osmotically active driving compound are also suitable for orally administered compounds. In these later platforms, fluid from the environment surrounding the capsule is imbibed by the driving compound, which swells to displace the agent or agent composition through an aperture. These delivery platforms can provide an essentially zero order delivery profile as opposed to the spiked profiles of immediate release formulations. A time-delay material such as glycerol monostearate or glycerol stearate can also be used.

The compositions can be intended for topical administration, in which case the carrier may be in the form of a solution, emulsion, ointment or gel base. If intended for transdermal administration, the composition can be in the form of a transdermal patch or an iontophoresis device. Topical formulations can comprise a concentration of an Exemplary Compound and/or Exemplary Conjugate of from about 0.05% to about 50% w/v (weight per unit volume of composition), in another aspect, from 0.1% to 10% w/v.

The composition can be intended for rectal administration, in the form, e.g., of a suppository which will melt in the rectum and release the Exemplary Compound and/or Exemplary Conjugate.

The composition can include various materials that modify the physical form of a solid or liquid dosage unit. For example, the composition can include materials that form a coating shell around the active ingredients. The materials that form the coating shell are typically inert, and can be selected from, for example, sugar, shellac, and other enteric coating agents. Alternatively, the active ingredients can be encased in a gelatin capsule.

The compositions can consist of gaseous dosage units, e.g., it can be in the form of an aerosol. The term aerosol is used to denote a variety of systems ranging from those of colloidal nature to systems consisting of pressurized packages. Delivery can be by a liquefied or compressed gas or by a suitable pump system that dispenses the active ingredients.

Whether in solid, liquid or gaseous form, the present compositions can include a pharmacological agent used in the treatment of cancer, an autoimmune disease or an infectious disease.

9.8 Therapeutic Uses of the Exemplary Conjugates

The Exemplary Compounds and/or Exemplary Conjugates are useful for treating cancer, an autoimmune disease or an infectious disease in a patient.

9.8.1 Treatment of Cancer

The Exemplary Compounds and/or Exemplary Conjugates are useful for inhibiting the multiplication of a tumor cell or cancer cell, causing apoptosis in a tumor or cancer cell, or for treating cancer in a patient. The Exemplary Compounds and/or Exemplary Conjugates can be used accordingly in a variety of settings for the treatment of animal cancers. The Drug-Linker-Ligand Conjugates can be

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used to deliver a Drug or Drug unit to a tumor cell or cancer cell. Without being bound by theory, in one embodiment, the Ligand unit of an Exemplary Conjugate binds to or associates with a cancer-cell or a tumor-cell-associated antigen, and the Exemplary Conjugate can be taken up inside a tumor cell or cancer cell through receptor-mediated endocytosis. The antigen can be attached to a tumor cell or cancer cell or can be an extracellular matrix protein associated with the tumor cell or cancer cell. Once inside the cell, one or more specific peptide sequences within the Linker unit are hydrolytically cleaved by one or more tumor-cell or cancer-cell-associated proteases, resulting in release of a Drug or a Drug-Linker Compound. The released Drug or Drug-Linker Compound is then free to migrate within the cell and induce cytotoxic or cytostatic activities. In an alternative embodiment, the Drug or Drug unit is cleaved from the Exemplary Conjugate outside the tumor cell or cancer cell, and the Drug or Drug-Linker Compound subsequently penetrates the cell.

In one embodiment, the Ligand unit binds to the tumor cell or cancer cell.

In another embodiment, the Ligand unit binds to a tumor cell or cancer cell antigen which is on the surface of the tumor cell or cancer cell.

In another embodiment, the Ligand unit binds to a tumor cell or cancer cell antigen which is an extracellular matrix protein associated with the tumor cell or cancer cell.

The specificity of the Ligand unit for a particular tumor cell or cancer cell can be important for determining those tumors or cancers that are most effectively treated. For example, Exemplary Conjugates having a BR96 Ligand unit can be useful for treating antigen positive carcinomas including those of the lung, breast, colon, ovaries, and pancreas. Exemplary Conjugates having an Anti-CD30 or an anti-CD40 Ligand unit can be useful for treating hematologic malignancies.

Other particular types of cancers that can be treated with Exemplary Conjugates include, but are not limited to, those disclosed in Table 3.

TABLE 3

Solid tumors, including but not limited to:	
fibrosarcoma	
myxosarcoma	
liposarcoma	
chondrosarcoma	
osteogenic sarcoma	
chordoma	
angiosarcoma	
endotheliosarcoma	
lymphangiosarcoma	
lymphangioendotheliosarcoma	
synovioma	
mesothelioma	
Ewing's tumor	
leiomyosarcoma	
rhabdomyosarcoma	
colon cancer	
colorectal cancer	
kidney cancer	
pancreatic cancer	
bone cancer	
breast cancer	
ovarian cancer	
prostate cancer	
esophageal cancer	
stomach cancer	
oral cancer	
nasal cancer	
throat cancer	
squamous cell carcinoma	

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TABLE 3-continued

basal cell carcinoma	
adenocarcinoma	
sweat gland carcinoma	
sebaceous gland carcinoma	
papillary carcinoma	
papillary adenocarcinomas	
cystadenocarcinoma	
medullary carcinoma	
bronchogenic carcinoma	
renal cell carcinoma	
hepatoma	
bile duct carcinoma	
choriocarcinoma	
seminoma	
embryonal carcinoma	
Wilms' tumor	
cervical cancer	
uterine cancer	
testicular cancer	
small cell lung carcinoma	
bladder carcinoma	
lung cancer	
epithelial carcinoma	
glioma	
glioblastoma multiforme	
astrocytoma	
medulloblastoma	
craniopharyngioma	
ependymoma	
pinealoma	
hemangioblastoma	
acoustic neuroma	
oligodendroglioma	
meningioma	
skin cancer	
melanoma	
neuroblastoma	
retinoblastoma	
blood-borne cancers, including but not limited to:	
acute lymphoblastic leukemia "ALL"	
acute lymphoblastic B-cell leukemia	
acute lymphoblastic T-cell leukemia	
acute myeloblastic leukemia "AML"	
acute promyelocytic leukemia "APL"	
acute monoblastic leukemia	
acute erythroleukemic leukemia	
acute megakaryoblastic leukemia	
acute myelomonocytic leukemia	
acute nonlymphocytic leukemia	
acute undifferentiated leukemia	
chronic myelocytic leukemia "CML"	
chronic lymphocytic leukemia "CLL"	
hairy cell leukemia	
multiple myeloma	
acute and chronic leukemias:	
lymphoblastic	
myelogenous	
lymphocytic	
myelocytic leukemias	
Lymphomas:	
Hodgkin's disease	
non-Hodgkin's Lymphoma	
Multiple myeloma	
Waldenström's macroglobulinemia	
Heavy chain disease	
Polycythemia vera	

The Exemplary Conjugates provide conjugation-specific tumor or cancer targeting, thus reducing general toxicity of these compounds. The Linker units stabilize the Exemplary Conjugates in blood, yet are cleavable by tumor-specific proteases within the cell, liberating a Drug.

9.8.2 Multi-Modality Therapy for Cancer

Cancers, including, but not limited to, a tumor, metastasis, or other disease or disorder characterized by uncontrolled

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cell growth, can be treated or prevented by administration of an Exemplary Conjugate and/or an Exemplary Compound.

In other embodiments, methods for treating or preventing cancer are provided, including administering to a patient in need thereof an effective amount of an Exemplary Conjugate and a chemotherapeutic agent. In one embodiment the chemotherapeutic agent is that with which treatment of the cancer has not been found to be refractory. In another embodiment, the chemotherapeutic agent is that with which the treatment of cancer has been found to be refractory. The Exemplary Conjugates can be administered to a patient that has also undergone surgery as treatment for the cancer.

In one embodiment, the additional method of treatment is radiation therapy.

In a specific embodiment, the Exemplary Conjugate is administered concurrently with the chemotherapeutic agent or with radiation therapy. In another specific embodiment, the chemotherapeutic agent or radiation therapy is administered prior or subsequent to administration of an Exemplary Conjugates, in one aspect at least an hour, five hours, 12 hours, a day, a week, a month, in further aspects several months (e.g., up to three months), prior or subsequent to administration of an Exemplary Conjugate.

A chemotherapeutic agent can be administered over a series of sessions. Any one or a combination of the chemotherapeutic agents listed in Table 4 can be administered. With respect to radiation, any radiation therapy protocol can be used depending upon the type of cancer to be treated. For example, but not by way of limitation, x-ray radiation can be administered; in particular, high-energy megavoltage (radiation of greater than 1 MeV energy) can be used for deep tumors, and electron beam and orthovoltage x-ray radiation can be used for skin cancers. Gamma-ray emitting radioisotopes, such as radioactive isotopes of radium, cobalt and other elements, can also be administered.

Additionally, methods of treatment of cancer with an Exemplary Compound and/or Exemplary Conjugate are provided as an alternative to chemotherapy or radiation therapy where the chemotherapy or the radiation therapy has proven or can prove too toxic, e.g., results in unacceptable or unbearable side effects, for the subject being treated. The animal being treated can, optionally, be treated with another cancer treatment such as surgery, radiation therapy or chemotherapy, depending on which treatment is found to be acceptable or bearable.

The Exemplary Compounds and/or Exemplary Conjugates can also be used in an in vitro or ex vivo fashion, such as for the treatment of certain cancers, including, but not limited to leukemias and lymphomas, such treatment involving autologous stem cell transplants. This can involve a multi-step process in which the animal's autologous hematopoietic stem cells are harvested and purged of all cancer cells, the animal's remaining bone-marrow cell population is then eradicated via the administration of a high dose of an Exemplary Compound and/or Exemplary Conjugate with or without accompanying high dose radiation therapy, and the stem cell graft is infused back into the animal. Supportive care is then provided while bone marrow function is restored and the animal recovers.

9.8.3 Multi-Drug Therapy for Cancer

Methods for treating cancer including administering to a patient in need thereof an effective amount of an Exemplary Conjugate and another therapeutic agent that is an anti-cancer agent are disclosed. Suitable anticancer agents include, but are not limited to, methotrexate, taxol, L-asparaginase, mercaptopurine, thioguanine, hydroxyurea, cytarabine, cyclophosphamide, ifosfamide, nitrosoureas, cis-

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platin, carboplatin, mitomycin, dacarbazine, procarbazine, topotecan, nitrogen mustards, cytoxan, etoposide, 5-fluorouracil, BCNU, irinotecan, camptothecins, bleomycin, doxorubicin, idarubicin, daunorubicin, dactinomycin, plicamycin, mitoxantrone, asparaginase, vinblastine, vincristine, vinorelbine, paclitaxel, and docetaxel. In one aspect, the anti-cancer agent includes, but is not limited to, a drug listed in Table 4.

TABLE 4

Alkylating agents	
Nitrogen mustards:	cyclophosphamide ifosfamide trofosfamide chlorambucil melphalan carmustine (BCNU) lomustine (CCNU)
Nitrosoureas:	
Alkylsulphonates	busulfan treosulfan
Triazines:	decabazine
Platinum containing compounds:	cisplatin carboplatin
Plant Alkaloids	
Vinca alkaloids:	vincristine vinblastine vindesine vinorelbine paclitaxel docetaxol
Taxoids:	
DNA Topoisomerase Inhibitors	
Epipodophyllins:	etoposide teniposide topotecan 9-aminocamptothecin camptothecin crisnatol mitomycin C
mitomycins:	
Anti-metabolites	
Anti-folates:	
DHFR inhibitors:	methotrexate trimetrexate
IMP dehydrogenase Inhibitors:	mycophenolic acid tiagofurin ribavirin EICAR
Ribonucleotide reductase Inhibitors:	hydroxyurea deferroxamine
Pyrimidine analogs:	
Uracil analogs	5-Fluorouracil floxuridine doxifluridine ratitrexed
Cytosine analogs	cytarabine (ara C) cytosine arabinoside fludarabine mercaptopurine thioguanine
Purine analogs:	
Hormonal therapies:	
Receptor antagonists:	
Anti-estrogen	tamoxifen raloxifene megestrol goserelin leuprolide acetate flutamide bicalutamide
LHRH agonists:	
Anti-androgens:	
Retinoids/Deltoids	
Vitamin D3 analogs:	EB 1089 CB 1093 KH 1060

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TABLE 4-continued

Alkylating agents	
Photodynamic therapies:	vertoporphin (BPD-MA) phthalocyanine photosensitizer Pc4 demethoxy-hypocrellin A (2BA-2-DMHA)
Cytokines:	Interferon- α Interferon- γ tumor necrosis factor
Others:	Gemcitabine Velcade Revamid Thalamid Lovastatin
Isoprenylation inhibitors:	1-methyl-4-phenylpyridinium ion
Dopaminergic neurotoxins:	staurosporine
Cell cycle inhibitors:	Actinomycin D
Actinomycins:	dactinomycin
Bleomycins:	bleomycin A2 bleomycin B2 peplomycin
Anthracyclines:	daunorubicin Doxorubicin (adriamycin) idarubicin epirubicin pirarubicin zorubicin
MDR inhibitors:	mtoxantrone
Ca ²⁺ ATPase inhibitors:	verapamil thapsigargin

9.8.4 Treatment of Autoimmune Diseases

The Exemplary Conjugates are useful for killing or inhibiting the replication of a cell that produces an autoimmune disease or for treating an autoimmune disease. The Exemplary Conjugates can be used accordingly in a variety of settings for the treatment of an autoimmune disease in a patient. The Drug-Linker-Ligand Conjugates can be used to deliver a Drug to a target cell. Without being bound by theory, in one embodiment, the Drug-Linker-Ligand Conjugate associates with an antigen on the surface of a target cell, and the Exemplary Conjugate is then taken up inside a target-cell through receptor-mediated endocytosis. Once inside the cell, one or more specific peptide sequences within the Linker unit are enzymatically or hydrolytically cleaved, resulting in release of a Drug. The released Drug is then free to migrate in the cytosol and induce cytotoxic or cytostatic activities. In an alternative embodiment, the Drug is cleaved from the Exemplary Conjugate outside the target cell, and the Drug subsequently penetrates the cell.

In one embodiment, the Ligand unit binds to an autoimmune antigen. In one aspect, the antigen is on the surface of a cell involved in an autoimmune condition.

In another embodiment, the Ligand unit binds to an autoimmune antigen which is on the surface of a cell.

In one embodiment, the Ligand binds to activated lymphocytes that are associated with the autoimmune disease state.

In a further embodiment, the Exemplary Conjugates kill or inhibit the multiplication of cells that produce an autoimmune antibody associated with a particular autoimmune disease.

Particular types of autoimmune diseases that can be treated with the Exemplary Conjugates include, but are not limited to, Th2 lymphocyte related disorders (e.g., atopic dermatitis, atopic asthma, rhinoconjunctivitis, allergic rhinitis, Omenn's syndrome, systemic sclerosis, and graft versus host disease); Th1 lymphocyte-related disorders (e.g., rheumatoid arthritis, multiple sclerosis, psoriasis, Sjorgren's

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syndrome, Hashimoto's thyroiditis, Grave's disease, primary biliary cirrhosis, Wegener's granulomatosis, and tuberculosis); activated B lymphocyte-related disorders (e.g., systemic lupus erythematosus, Goodpasture's syndrome, rheumatoid arthritis, and type I diabetes); and those disclosed in Table 5.

TABLE 5

Active Chronic Hepatitis
Addison's Disease
Allergic Alveolitis
Allergic Reaction
Allergic Rhinitis
Alport's Syndrome
Anaphylaxis
Ankylosing Spondylitis
Anti-phospholipid Syndrome
Arthritis
Ascariasis
Aspergillosis
Atopic Allergy
Atopic Dermatitis
Atropic Rhinitis
Behcet's Disease
Bird-Fancier's Lung
Bronchial Asthma
Caplan's Syndrome
Cardiomyopathy
Celiac Disease
Chagas' Disease
Chronic Glomerulonephritis
Cogan's Syndrome
Cold Agglutinin Disease
Congenital Rubella Infection
CREST Syndrome
Crohn's Disease
Cryoglobulinemia
Cushing's Syndrome
Dermatomyositis
Discoid Lupus
Dressler's Syndrome
Eaton-Lambert Syndrome
Echovirus Infection
Encephalomyelitis
Endocrine ophthalmopathy
Epstein-Barr Virus Infection
Equine Heaves
Erythematosis
Evan's Syndrome
Felty's Syndrome
Fibromyalgia
Fuch's Cyclitis
Gastric Atrophy
Gastrointestinal Allergy
Giant Cell Arteritis
Glomerulonephritis
Goodpasture's Syndrome
Graft v. Host Disease
Graves' Disease
Guillain-Barre Disease
Hashimoto's Thyroiditis
Hemolytic Anemia
Henoch-Schonlein Purpura
Idiopathic Adrenal Atrophy
Idiopathic Pulmonary Fibrosis
IgA Nephropathy
Inflammatory Bowel Diseases
Insulin-dependent Diabetes Mellitus
Juvenile Arthritis
Juvenile Diabetes Mellitus (Type I)
Lambert-Eaton Syndrome
Laminitis
Lichen Planus
Lupoid Hepatitis
Lupus
Lymphopenia
Meniere's Disease
Mixed Connective Tissue Disease
Multiple Sclerosis

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TABLE 5-continued

Myasthenia Gravis	
Pernicious Anemia	
Polyglandular Syndromes	
Presenile Dementia	
Primary Agammaglobulinemia	
Primary Biliary Cirrhosis	
Psoriasis	
Psoriatic Arthritis	
Raynauds Phenomenon	
Recurrent Abortion	
Reiter's Syndrome	10
Rheumatic Fever	
Rheumatoid Arthritis	
Sampter's Syndrome	
Schistosomiasis	
Schmidt's Syndrome	
Scleroderma	15
Shulman's Syndrome	
Sjorgen's Syndrome	
Stiff-Man Syndrome	
Sympathetic Ophthalmia	
Systemic Lupus Erythematosus	
Takayasu's Arteritis	20
Temporal Arteritis	
Thyroiditis	
Thrombocytopenia	
Thyrotoxicosis	
Toxic Epidermal Necrolysis	
Type B Insulin Resistance	25
Type I Diabetes Mellitus	
Ulcerative Colitis	
Uveitis	
Vitiligo	
Waldenstrom's Macroglobulemia	
Wegener's Granulomatosis	30

9.8.5 Multi-Drug Therapy of Autoimmune Diseases

Methods for treating an autoimmune disease are also disclosed including administering to a patient in need thereof an effective amount of an Exemplary Conjugate and another therapeutic agent known for the treatment of an autoimmune disease. In one embodiment, the anti-autoimmune disease agent includes, but is not limited to, agents listed in Table 6.

TABLE 6

cyclosporine	
cyclosporine A	
mycophenylate mofetil	
sirolimus	45
tacrolimus	
enanercept	
prednisone	
azathioprine	
methotrexate cyclophosphamide	50
prednisone	
aminocaproic acid	
chloroquine	
hydroxychloroquine	
hydrocortisone	
dexamethasone	55
chlorambucil	
DHEA	
danazol	
bromocriptine	
meloxicam	
infliximab	60

9.8.6 Treatment of Infectious Diseases

The Exemplary Conjugates are useful for killing or inhibiting the multiplication of a cell that produces an infectious disease or for treating an infectious disease. The Exemplary Conjugates can be used accordingly in a variety of settings for the treatment of an infectious disease in a patient. The

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Drug-Linker-Ligand Conjugates can be used to deliver a Drug to a target cell. In one embodiment, the Ligand unit binds to the infectious disease cell.

In one embodiment, the Conjugates kill or inhibit the multiplication of cells that produce a particular infectious disease.

Particular types of infectious diseases that can be treated with the Exemplary Conjugates include, but are not limited to, those disclosed in Table 7.

TABLE 7

Bacterial Diseases:	
Diphtheria	
Pertussis	
Occult Bacteremia	
Urinary Tract Infection	
Gastroenteritis	
Cellulitis	
Epiglottitis	
Tracheitis	
Adenoid Hypertrophy	
Retropharyngeal Abscess	
Impetigo	
Ecthyma	
Pneumonia	
Endocarditis	
Septic Arthritis	
Pneumococcal	
Peritonitis	
Bacteremia	
Meningitis	
Acute Purulent Meningitis	
Urethritis	
Cervicitis	
Proctitis	
Pharyngitis	
Salpingitis	
Epididymitis	
Gonorrhea	35
Syphilis	
Listeriosis	
Anthrax	
Nocardiosis	
<i>Salmonella</i>	
Typhoid Fever	
Dysentery	40
Conjunctivitis	
Sinusitis	
Brucellosis	
Tularemia	
Cholera	
Bubonic Plague	45
Tetanus	
Necrotizing Enteritis	
Actinomycosis	
Mixed Anaerobic Infections	
Syphilis	
Relapsing Fever	50
Leptospirosis	
Lyme Disease	
Rat Bite Fever	
Tuberculosis	
Lymphadenitis	
Leprosy	55
Chlamydia	
Chlamydial Pneumonia	
Trachoma	
Inclusion Conjunctivitis	
Systemic Fungal Diseases:	
Histoplasmosis	
Coccidioidomycosis	
Blastomycosis	
Sporotrichosis	
Cryptococcosis	
Systemic Candidiasis	
Aspergillosis	
Mucormycosis	

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TABLE 7-continued

Mycetoma	
Chromomycosis	
Rickettsial Diseases:	
Typhus	5
Rocky Mountain Spotted Fever	
Ehrlichiosis	
Eastern Tick-Borne Rickettsioses	
Rickettsialpox	
Q Fever	10
Bartonellosis	
Parasitic Diseases:	
Malaria	
Babesiosis	
African Sleeping Sickness	15
Chagas' Disease	
Leishmaniasis	
Dum-Dum Fever	
Toxoplasmosis	
Meningoencephalitis	
Keratitis	
Entamebiasis	20
Giardiasis	
Cryptosporidiasis	
Isosporiasis	
Cyclosporiasis	
Microsporidiosis	
Ascariasis	25
Whipworm Infection	
Hookworm Infection	
Threadworm Infection	
Ocular Larva Migrans	
Trichinosis	30
Guinea Worm Disease	
Lymphatic Filariasis	
Loiasis	
River Blindness	
Canine Heartworm Infection	
Schistosomiasis	
Swimmer's Itch	35
Oriental Lung Fluke	
Oriental Liver Fluke	
Fascioliasis	
Fasciolopsiasis	
Opisthorchiasis	
Tapeworm Infections	40
Hydatid Disease	
Alveolar Hydatid Disease	
Viral Diseases:	
Measles	
Subacute sclerosing panencephalitis	
Common Cold	45
Mumps	
Rubella	
Roseola	
Fifth Disease	
Chickenpox	
Respiratory syncytial virus infection	50
Croup	
Bronchiolitis	
Infectious Mononucleosis	
Poliomyelitis	
Herpangina	
Hand-Foot-and-Mouth Disease	55
Bornholm Disease	
Genital Herpes	
Genital Warts	
Aseptic Meningitis	
Myocarditis	
Pericarditis	
Gastroenteritis	60
Acquired Immunodeficiency Syndrome (AIDS)	
Human Immunodeficiency Virus (HIV)	
Reye's Syndrome	
Kawasaki Syndrome	
Influenza	
Bronchitis	65
Viral "Walking" Pneumonia	

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TABLE 7-continued

Acute Febrile Respiratory Disease
Acute pharyngoconjunctival fever
Epidemic keratoconjunctivitis
Herpes Simplex Virus 1 (HSV-1)
Herpes Simplex Virus 2 (HSV-2)
Shingles
Cytomegalic Inclusion Disease
Rabies
Progressive Multifocal Leukoencephalopathy
Kuru
Fatal Familial Insomnia
Creutzfeldt-Jakob Disease
Gerstmann-Straussler-Scheinker Disease
Tropical Spastic Paraparesis
Western Equine Encephalitis
California Encephalitis
St. Louis Encephalitis
Yellow Fever
Dengue
Lymphocytic choriomeningitis
Lassa Fever
Hemorrhagic Fever
Hantavirus Pulmonary Syndrome
Marburg Virus Infections
Ebola Virus Infections
Smallpox

9.8.7 Multi-Drug Therapy of Infectious Diseases

Methods for treating an infectious disease are disclosed including administering to a patient in need thereof an Exemplary Conjugate and another therapeutic agent that is an anti-infectious disease agent. In one embodiment, the anti-infectious disease agent is, but not limited to, agents listed in Table 8.

TABLE 8

	β -Lactam Antibiotics:
	Penicillin G
	Penicillin V
	Cloxacillin
	Dicloxacillin
	Methicillin
	Nafcillin
	Oxacillin
	Ampicillin
	Amoxicillin
	Bacampicillin
	Azlocillin
	Carbenicillin
	Mezlocillin
	Piperacillin
	Ticarcillin
	Aminoglycosides:
	Amikacin
	Gentamicin
	Kanamycin
	Neomycin
	Netilmicin
	Streptomycin
	Tobramycin
	Macrolides:
	Azithromycin
	Clarithromycin
	Erythromycin
	Lincomycin
	Clindamycin
	Tetracyclines:
	Demeclocycline
	Doxycycline
	Minocycline
	Oxytetracycline
	Tetracycline

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TABLE 8-continued

Quinolones:	
Cinoxacin	5
Nalidixic Acid	
Fluoroquinolones:	
Ciprofloxacin	10
Enoxacin	
Grepafloxacin	
Levofloxacin	
Lomefloxacin	
Norfloxacin	
Ofloxacin	
Sparfloxacin	
Trovafoxacin	15
Polypeptides:	
Bacitracin	
Colistin	20
Polymyxin B	
Sulfonamides:	
Sulfisoxazole	
Sulfamethoxazole	25
Sulfadiazine	
Sulfamethizole	
Sulfacetamide	
Miscellaneous Antibacterial Agents:	25
Trimethoprim	
Sulfamethazole	
Chloramphenicol	
Vancomycin	

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TABLE 8-continued

Penciclovir
Valacyclovir
Ganciclovir
Foscarnet
Ribavirin
Amantadine
Rimantadine
Cidofovir
Antisense Oligonucleotides
Immunoglobulins
Interferons
Drugs for HIV infection:
Tenofovir
Emtricitabine
Zidovudine
Didanosine
Zalcitabine
Stavudine
Lamivudine
Nevirapine
Delavirdine
Saquinavir
Ritonavir
Indinavir
Nelfinavir

EXAMPLES

Example 1—Preparation of Compound AB

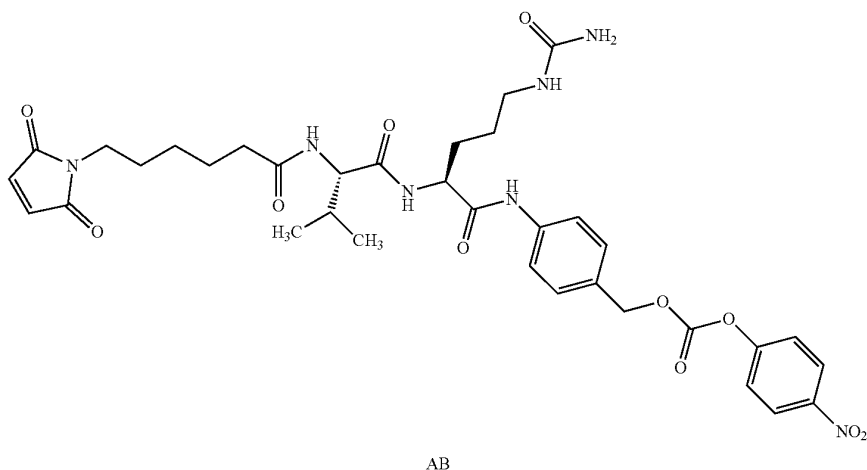


TABLE 8-continued

Metronidazole
Quinupristin
Dalfopristin
Rifampin
Spectinomycin
Nitrofurantoin
Antiviral Agents:
General Antiviral Agents:
Idoxuradine
Vidarabine
Trifluridine
Acyclovir
Famciclovir

55 Fmoc-val-cit-PAB-OH (14.61 g, 24.3 mmol, 1.0 eq., U.S. Pat. No. 6,214,345 to Firestone et al.) was diluted with DMF (120 mL, 0.2 M) and to this solution was added a diethylamine (60 mL).

The reaction was monitored by HPLC and found to be complete in 2 h. The reaction mixture was concentrated and the resulting residue was precipitated using ethyl acetate (ca. 100 mL) under sonication over for 10 min. Ether (200 mL) was added and the precipitate was further sonicated for 5 min. The solution was allowed to stand for 30 min. without stirring and was then filtered and dried under high vacuum to provide Val-cit-PAB-OH, which was used in the next step without further purification. Yield: 8.84 g (96%). Val-cit-PAB-OH (8.0 g, 21 mmol) was diluted with DMF (110 mL)

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and the resulting solution was treated with MC-OSu (Willner et al., (1993) Bioconjugate Chem. 4:521; 6.5 g, 21 mmol, 1.0 eq.). Reaction was complete according to HPLC after 2 h. The reaction mixture was concentrated and the resulting oil was precipitated using ethyl acetate (50 mL). After sonicating for 15 min, ether (400 mL) was added and the mixture was sonicated further until all large particles were broken up. The solution was then filtered and the solid dried to provide an off-white solid intermediate. Yield: 11.63 g (96%); ES-MS m/z 757.9 [M-H]

Fmoc-val-cit-PAB-OH (14.61 g, 24.3 mmol, 1.0 eq., U.S. Pat. No. 6,214,345 to Firestone et al.) was diluted with DMF (120 mL, 0.2 M) and to this solution was added a diethylamine (60 mL). The reaction was monitored by HPLC and found to be complete in 2 h. The reaction mixture was concentrated and the resulting residue was precipitated using ethyl acetate (ca. 100 mL) under sonication over for 10 min. Ether (200 mL) was added and the precipitate was further sonicated for 5 min. The solution was allowed to stand for 30 min. without stirring and was then filtered and dried under high vacuum to provide Val-cit-PAB-OH, which was used in the next step without further purification. Yield: 8.84 g (96%). Val-cit-PAB-OH (8.0 g, 21 mmol) was diluted with DMF (110 mL) and the resulting solution was treated with MC-OSu (Willner et al., (1993) Bioconjugate Chem. 4:521; 6.5 g, 21 mmol, 1.0 eq.). Reaction was complete according to HPLC after 2 h. The reaction mixture was concentrated and the resulting oil was precipitated using ethyl acetate (50 mL). After sonicating for 15 min, ether (400 mL) was added and the mixture was sonicated further until all large particles were broken up. The solution was then filtered and the solid dried to provide an off-white solid intermediate. Yield: 11.63 g (96%); ES-MS m/z 757.9 [M-H].

The off-white solid intermediate (8.0 g, 14.0 mmol) was diluted with DMF (120 mL, 0.12 M) and to the resulting solution was added bis(4-nitrophenyl)carbonate (8.5 g, 28.0 mmol, 2.0 eq.) and DIEA (3.66 mL, 21.0 mmol, 1.5 eq.). The reaction was complete in 1 h according to HPLC. The reaction mixture was concentrated to provide an oil that was precipitated with EtOAc, and then triturated with EtOAc (ca. 25 mL). The solute was further precipitated with ether (ca. 200 mL) and triturated for 15 min. The solid was filtered and dried under high vacuum to provide Compound AB which was 93% pure according to HPLC and used in the next step without further purification. Yield: 9.7 g (94%).

Example 2—Preparation of Compound 1

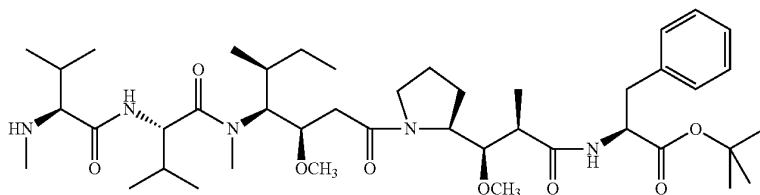
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Phenylalanine t-butyl ester HCl salt (868 mg, 3 mmol), N-Boc-Dolaproine (668 mg, 1 eq.), DEPC (820 μ L, 1.5 eq.), and DIEA (1.2 mL) were diluted with dichloromethane (3 mL). After 2 hours (h) at room temperature (about 28 degrees Celsius), the reaction mixture was diluted with dichloromethane (20 mL), washed successively with saturated aqueous (aq.) NaHCO_3 (2 \times 10 mL), saturated aq. NaCl (2 \times 10 mL). The organic layer was separated and concentrated. The resulting residue was re-suspended in ethyl acetate and was purified via flash chromatography in ethyl acetate. The relevant fractions were combined and concentrated to provide the dipeptide as a white solid: 684 mg (46%). ES-MS m/z 491.3 [M+H]⁺.

For selective Boc cleavage in the presence of t-butyl ester, the above dipeptide (500 mg, 1.28 mmol) was diluted with dioxane (2 mL). 4M HCl/dioxane (960 μ L, 3 eq.) was added, and the reaction mixture was stirred overnight at room temperature. Almost complete Boc deprotection was observed by RP-HPLC with minimal amount of t-butyl ester cleavage. The mixture was cooled down on an ice bath, and triethylamine (500 μ L) was added. After 10 min., the mixture was removed from the cooling bath, diluted with dichloromethane (20 mL), washed successively with saturated aq. NaHCO_3 (2 \times 10 mL), saturated aq. NaCl (2 \times 10 mL). The organic layer was concentrated to give a yellow foam: 287 mg (57%). The intermediate was used without further purification.

The tripeptide Fmoc-Meval-val-dil-O-t-Bu (prepared as described in WO 02/088172, entitled "Pentapeptide Compounds and Uses Related Thereto"; 0.73 mmol) was treated with TFA (3 mL), dichloromethane (3 mL) for 2 h at room temperature. The mixture was concentrated to dryness, the residue was co-evaporated with toluene (3 \times 20 mL), and dried in vacuum overnight. The residue was diluted with dichloromethane (5 mL) and added to the deprotected dipeptide (287 mg, 0.73 mmol), followed by DIEA (550 μ L, 4 eq.), DEPC (201 μ L, 1.1 eq.). After 2 h at room temperature the reaction mixture was diluted with ethyl acetate (50 mL), washed successively with 10% aq. citric acid (2 \times 20 mL), saturated aq. NaHCO_3 (2 \times 10 mL), saturated aq. NaCl (10 mL). The organic layer was separated and concentrated. The resulting residue was re-suspended in ethyl acetate and was purified via flash chromatography in ethyl acetate. The relevant fractions were combined and concentrated to provide Fmoc-Meval-val-dil-dap-phe-O-t-Bu as a white solid: 533 mg (71%). R_f 0.4 (EtOAc). ES-MS m/z 1010.6 [M+H]⁺

The product (200 mg, 0.2 mmol) was diluted with dichloromethane (3 mL), diethylamine (1 mL). The reaction mixture was stirred overnight at room temperature. Solvents were removed to provide an oil that was purified by flash silica gel chromatography in a step gradient 0-10% MeOH in dichloromethane to provide Compound 1 as a white solid: 137 mg (87%). R_f 0.3 (10% MeOH/ CH_2Cl_2). ES-MS m/z 788.6 [M+H]⁺.

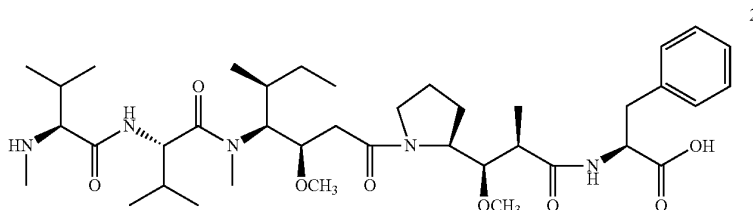


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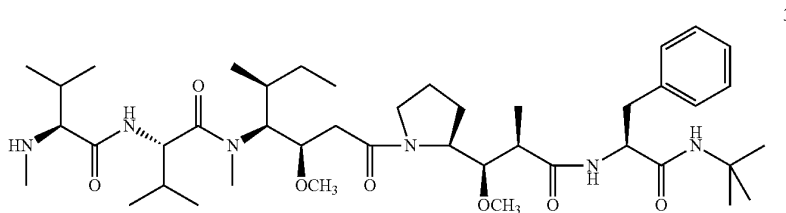
Example 3—Preparation of Compound 2

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Compound 2 was prepared from compound 1 (30 mg, 0.038 mmol) by treatment with 4M HCl/dioxane (4 ml) for 7 h at room temperature. The solvent was removed, and the residue was dried in a vacuum overnight to give provide Compound 2 as a hygroscopic white solid: 35 mg (120% calculated for HCl salt). ES-MS m/z 732.56 $[M+H]^+$

Example 4—Preparation of Compound 3



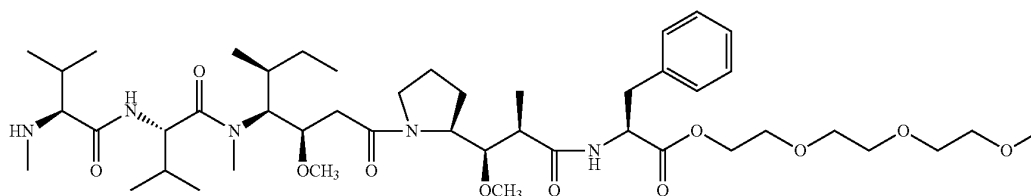
Fmoc-Meal-val-dil-dap-phe-O-t-Bu (Example 2, 50 mg) was treated with 4M HCl/dioxane (4 ml) for 16 h at room temperature. The solvent was removed, and the residue was dried in vacuum overnight to give 50 mg of a hygroscopic white solid intermediate

organic layer was separated and concentrated. The resulting residue was diluted with dichloromethane and purified via flash chromatography in a step gradient 0-5% MeOH in dichloromethane. The relevant fractions were combined and concentrated to provide the Fmoc protected intermediate as a white solid: 7.3 mg (36%). R_f 0.75 (10% MeOH/ CH_2Cl_2).

The white solid intermediate (20 mg, 0.02 mmol) was diluted with dichloromethane (1 mL); DEPC (5 μ L, 0.03 mmol, 1.5 eq.) was added followed by DIEA (11 μ L, 0.06 mmol, 3 eq.), and t-butylamine (3.2 μ L, 0.03 mmol, 1.5 eq.). After 2 h at room temperature, the reaction was found to be uncompleted by RP-HPLC. More DEPC (10 μ L) and t-butylamine (5 μ L) were added and the reaction was stirred for additional 4 h. Reaction mixture was diluted with dichloromethane (15 mL), washed successively with water (5 mL), 0.1 M aq. HCl (10 mL), saturated aq. NaCl (10 mL). The

Fmoc protected intermediate was diluted with dichloromethane (0.5 mL) and treated with diethylamine (0.5 mL) for 3 h at room temperature. The reaction mixture was concentrated to dryness. The product was isolated by flash silica gel chromatography in a step gradient 0-10% MeOH in dichloromethane to provide Compound 3 as a white solid: 4 mg (70%). R_f 0.2 (10% MeOH/ CH_2Cl_2). ES-MS m/z 787 $[M+H]^+$, 809 $[M+Na]^+$.

Example 5—Preparation of Compound 4



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Boc-L-Phenylalanine (265 mg, 1 mmol, 1 eq.) and triethyleneglycol monomethyl ether (164 μ L, 1 mmol, 1 eq.) were diluted with dichloromethane (5 mL). Then, DCC (412 mg, 2 mmol, 2 eq.) was added, followed by DMAP (10 mg). The reaction mixture was stirred overnight at room temperature. The precipitate was filtered off. The solvent was removed in a vacuum, the residue was diluted with ethyl acetate, and purified by silica gel flash chromatography in ethyl acetate. The product containing fractions were pulled, concentrated, and dried in vacuum to give a white solid: 377 mg (91%). R_f 0.5 (EtOAc). ES-MS m/z 434 $[M+Na]^+$.

Removal of Boc protecting group was performed by treatment of the above material in dioxane (10 mL) with 4M HCl/dioxane (6 mL) for 6 h at room temperature. The solvent was removed in a vacuum, the residue was dried in a vacuum to give a white solid.

The HCl salt of Phenylalanine-triethyleneglycol monomethyl ether ester (236 mg, 0.458 mmol, 1 eq.) and N-Boc-Dolaproine (158 mg, 0.55 mmol, 1.2 eq.) were diluted with dichloromethane (3 mL). DEPC (125 μ L, 1.5 eq.) and added to the mixture followed by DIEA (250 μ L, 3 eq.). After 2 h at room temperature the reaction mixture was diluted with ethyl acetate (30 mL), washed successively with saturated aq. $NaHCO_3$ (2 \times 10 mL), 10% aq. citric acid (2 \times 10 mL), saturated aq. NaCl (10 mL). The organic layer was separated and concentrated. The resulting residue was re-suspended in ethyl acetate and was purified via flash chromatography on silica gel in ethyl acetate. The relevant fractions were combined and concentrated to provide a white foam intermediate: 131 mg (50%). R_f 0.25 (EtOAc). ES-MS m/z 581.3 $[M+H]^+$.

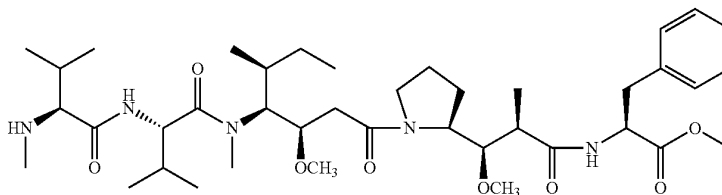
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Boc deprotection was done in dichloromethane (2 mL), TFA (0.5 mL) at room temperature for 2 h. Solvent was removed in vacuum, and the residue was co-evaporated with toluene (3 \times 25 mL), then dried in vacuum to give 138 mg of dipeptide TFA salt.

Fmoc-Meval-val-dil-OH (Example 2, 147 mg, 0.23 mmol, 1 eq.), and dipeptide TFA salt (138 mg) were diluted with dichloromethane (2 mL). To the mixture DEPC (63 μ L, 1.5 eq.) was added, followed by DIEA (160 μ L, 4 eq.). After 2 h at room temperature the reaction mixture was diluted with dichloromethane (30 mL), washed successively with 10% aq. citric acid (2 \times 20 mL), saturated aq. NaCl (20 mL). The organic layer was separated and concentrated. The resulting residue was re-suspended in dichloromethane and was purified via flash chromatography on silica gel in a step gradient 0-5% MeOH in dichloromethane. The relevant fractions were combined and concentrated to provide white foam: 205 mg (81%). R_f 0.4 (10% MeOH/ CH_2Cl_2). ES-MS m/z 1100.6 $[M+H]^+$, 1122.4 $[M+Na]^+$

Fmoc protecting group was removed by treatment with diethylamine (2 mL) in dichloromethane (6 mL). After 6 h at room temperature solvent was removed in vacuum, product was isolated by flash chromatography on silica gel in a step gradient 0-10% MeOH in dichloromethane. The relevant fractions were combined and concentrated. After evaporation from dichloromethane/hexane, 1:1, Compound 4 was obtained as a white foam: 133 mg (80%). R_f 0.15 (10% MeOH/ CH_2Cl_2). ES-MS m/z 878.6 $[M+H]^+$.

Example 6—Preparation of Compound 5



Fmoc-Meval-val-dil-OH (Example 2, 0.50 g, 0.78 mmol) and dap-phe-OMe.HCl (0.3 g, 0.78 mmol, prepared according to Pettit, G. R., et al. *Anti-Cancer Drug Design* 1998, 13, 243-277) were dissolved in CH_2Cl_2 (10 mL) followed by the addition of diisopropylethylamine (0.30 mL, 1.71 mmol, 2.2 eq.). DEPC (0.20 mL, 1.17, 1.5 eq.) was added and the contents stood over Ar. Reaction was complete according to HPLC in 1 h. The mixture was concentrated to an oil and purified by SiO_2 chromatography (300 \times 25 mm column) and eluting with 100% EtOAc. The product was isolated as a white foamy solid. Yield: 0.65 g (87%). ES-MS m/z 968.35 $[M+H]^+$, 991.34 $[M+Na]^+$; UV λ_{max} 215, 265 nm.

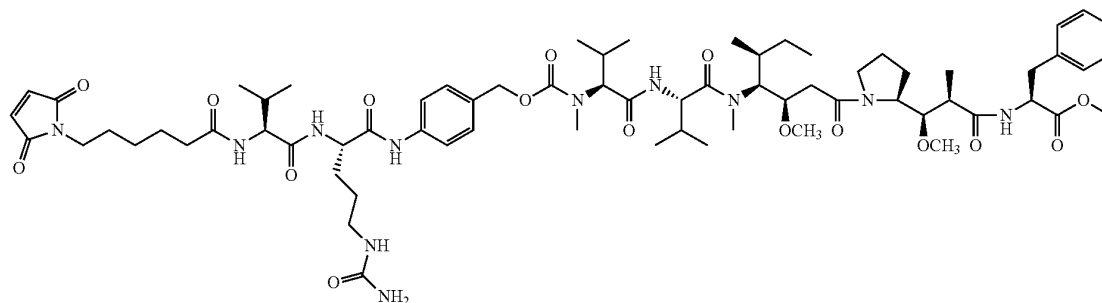
The Fmoc-protected peptide (0.14 g, 0.14 mmol) in methylene chloride (5 mL) was treated with diethylamine (2 mL) and the contents stood at room temperature for 2 h. The reaction, complete by HPLC, was concentrated to an oil, taken up in 2 mL of DMSO and injected into a preparative-HPLC (C_{12} -RP column, 5 μ , 100 Å, linear gradient of MeCN in water (containing 0.1% TFA) 10 to 100% in 40 min followed by 20 min at 100%, at a flow rate of 25 mL/min). Fractions containing the product were evaporated to afford a white powder for the trifluoroacetate salt. Yield: 0.126 g (98%). R_f 0.28 (100% EtOAc); ES-MS m/z 746.59 $[M+H]^+$, 768.51 $[M+Na]^+$; UV λ_{max} 215 nm.

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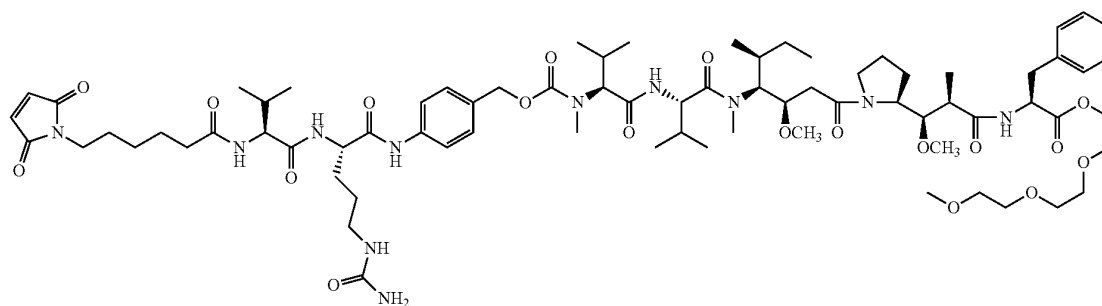
Example 7—Preparation of Compound 6



The trifluoroacetate salt of Compound 5 (0.11 g, 0.13 mmol), Compound AB (0.103 g, 0.14 mmol, 1.1 eq.) and HOBt (3.4 mg, 26 μ mol, 0.2 eq.) were suspended in DMF/pyridine (2 mL/0.5 mL, respectively). Diisopropylethylamine (22.5 μ L, 0.13 mmol, 1.0 eq.) was added and the yellow solution stirred while under argon. After 3 h, an additional 1.0 eq. of DIEA was added.

24 hours later, 0.5 eq. of the activated linker was included in the reaction mixture. After 40 h total, the reaction was complete. The contents were evaporated, taken up in DMSO and injected into a prep-HPLC (C_{12} -RP column, 5 μ , 100 \AA , linear gradient of MeCN in water (containing 0.1% TFA) 10 to 100% in 40 min followed by 20 min at 100%, at a flow rate of 50 mL/min). The desired fractions were evaporated to give the product as a yellow oil. Methylene chloride (ca. 2 mL) and excess ether were added to provide Compound 6 as a white precipitate that was filtered and dried. Yield: 90 mg (52%). ES-MS m/z 1344.32 $[M+H]^+$, 1366.29 $[M+Na]^+$; UV λ_{max} 215, 248 nm.

Example 8—Preparation of Compound 7



Compound 4 (133 mg, 0.15 mmol, 1 eq.), Compound AB, (123 mg, 0.167 mmol, 1.1 eq.), and HOBt (4 mg, 0.2 eq.) were diluted with DMF (1.5 mL). After 2 min, pyridine (5 mL) was added and the reaction was monitored using RP-HPLC. The reaction was shown to be complete within 18 h. The reaction mixture was diluted with dichloromethane (20 mL), washed successively with 10% aq. citric acid (2 \times 10 mL), water (10 mL), saturated aq. NaCl (10 mL). The

organic layer was separated and concentrated. The resulting residue was re-suspended in dichloromethane and was purified via flash chromatography on silica gel in a step gradient 0-10% MeOH in dichloromethane. The relevant fractions were combined and concentrated to provide Compound 7 as a white foam: 46 mg (21%). R_f 0.15 (10% MeOH/ CH_2Cl_2). ES-MS m/z 1476.94 $[M+H]^+$

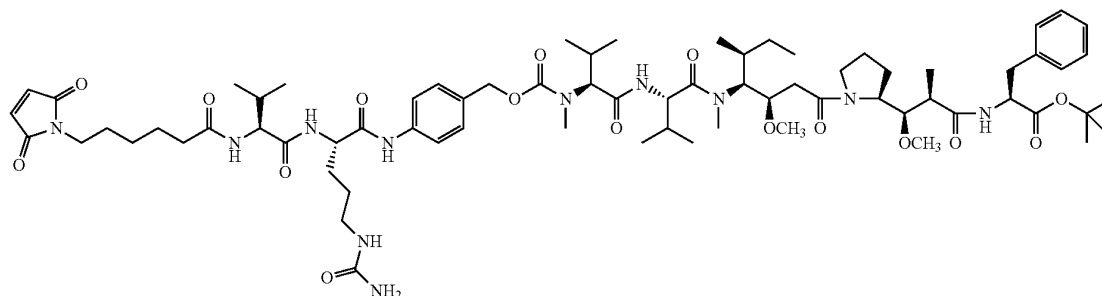
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Example 9—Preparation of
MC-Val-Cit-PAB-MMAF t-butyl ester 8

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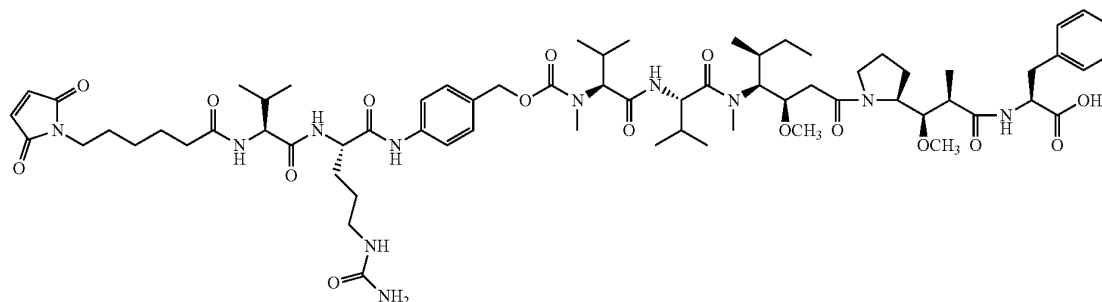
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Compound 1 (83 mg, 0.11 mmol), Compound AB (85 mg, 0.12 mmol, 1.1 eq.), and HOBT (2.8 mg, 21 μ mol, 0.2 eq.) were taken up in dry DMF (1.5 mL) and pyridine (0.3 mL) while under argon. After 30 h, the reaction was found to be essentially complete by HPLC. The mixture was evaporated, taken up in a minimal amount of DMSO and purified by prep-HPLC (C₁₂-RP column, 5 μ , 100 Å, linear gradient of MeCN in water (containing 0.1% TFA) 10 to 100% in 40 min followed by 20 min at 100%, at a flow rate of 25 mL/min) to provide Compound 8 as a white solid. Yield: 103 mg (71%). ES-MS m/z 1387.06 [M+H]⁺, 1409.04 [M+Na]⁺; UV λ_{max} 205, 248 nm.

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Example 10—Preparation of MC-val-cit-PAB-MMAF 9

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Compound 8 (45 mg, 32 μ mol) was suspended in methylene chloride (6 mL) followed by the addition of TFA (3 mL). The resulting solution stood for 2 h. The reaction mixture was concentrated in vacuo and purified by prep-HPLC (C_{12} -RP column, 5 μ , 100 Å, linear gradient of MeCN 60 in water (containing 0.1% TFA) 10 to 100% in 40 min followed by 20 min at 100%, at a flow rate of 25 mL/min). The desired fractions were concentrated to provide maleimido-caproyl-valine-citrulline-p-hydroxymethylaminobenzene-MMAF (MC-val-cit-PAB-MMAF) 9 as an off-white 65 solid. Yield: 11 mg (25%). ES-MS m/z 1330.29 [M+1-1]⁺, 1352.24 [M+Na]⁺; UV λ_{max} 205, 248 nm.

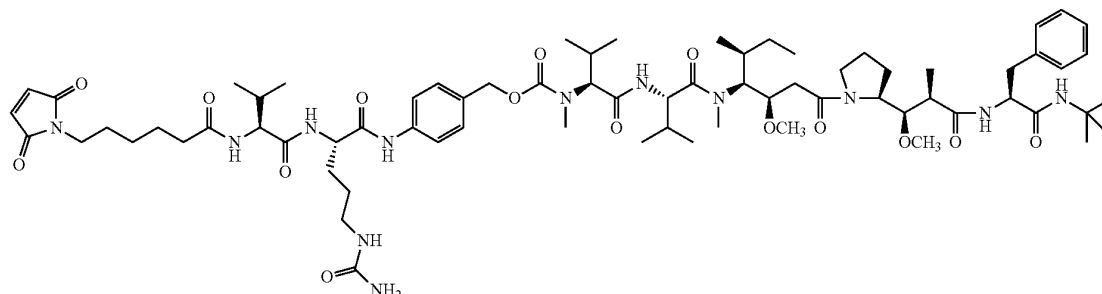
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Example 11—Preparation of
MC-val-cit-PAB-MMAF tert-butyl amide 10

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Compound 3 (217 mg, 0.276 mmol, 1.0 eq.), Compound AB (204 mg, 0.276 mmol, 1.0 eq.), and HOBt (11 mg, 0.0828 mmol, 0.3 eq.) were diluted with pyridine/DMF (6 mL). To this mixture was added DIEA (0.048 mL), and the mixture was stirred ca. 16 hr. Volatile organics were evaporated in vacuo. The crude residue was purified by Chromatotron® (radial thin-layer chromatography) with a step gradient (0-5-10% methanol in DCM) to provide MC-val-cit-PAB-MMAF tert-butyl amide 10. Yield: 172 mg (45%); ES-MS m/z 1386.33 $[M+H]^+$, 1408.36 $[M+Na]^+$; UV λ_{max} 215, 248 nm.

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Example 14—Preparation of
AC10-MC-val-cit-PAB-MMAE by Conjugation of
AC10 and MC-val-cit-PAB-MMAE

AC10-MC-val-cit-PAB-MMAE was prepared by conjugation of AC10 and MC-val-cit-PAB-MMAE following the procedure of Example 12.

Example 15—Preparation of AC10-MC-val-cit-PAB-MMAF by Conjugation of AC10 and MC-val-cit-PAB-MMAF (9)

AC10-MC-val-cit-PAB-MMAF was prepared by conjugation of AC10 and MC-val-cit-PAB-MMAF (9) following the procedure of Example 12.

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Example 16—Determination of Cytotoxicity of
Selected Compounds

AC10, dissolved in 500 mM sodium borate and 500 mM sodium chloride at pH 8.0 is treated with an excess of 100 mM dithiothreitol (DTT). After incubation at 37° C. for about 30 minutes, the buffer is exchanged by elution over Sephadex G25 resin and eluted with PBS with 1 mM DTPA. The thiol/Ab value is checked by determining the reduced antibody concentration from the absorbance at 280 nm of the solution and the thiol concentration by reaction with DTNB (Aldrich, Milwaukee, Wis.) and determination of the absorbance at 412 nm. The reduced antibody dissolved in PBS is chilled on ice.

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The drug linker reagent, maleimidocaproyl-monomethyl auristatin E, i.e. MC-MMAE, dissolved in DMSO, is diluted in acetonitrile and water at known concentration, and added to the chilled reduced antibody AC10 in PBS. After about one hour, an excess of maleimide is added to quench the reaction and cap any unreacted antibody thiol groups. The reaction mixture is concentrated by centrifugal ultrafiltration and AC10-MC-MMAE is purified and desalted by elution through G25 resin in PBS, filtered through 0.2 μ m filters under sterile conditions, and frozen for storage.

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Cytotoxic activity of MMAF and Compounds 1-5 was evaluated on the Lewis Y positive cell lines OVCAR-3, H3396 breast carcinoma, L2987 lung carcinoma and LS174t colon carcinoma Lewis Y positive cell lines can be assayed for cytotoxicity. To evaluate the cytotoxicity of Compounds 1-5, cells can be seeded at approximately 5-10,000 per well in 150 μ l of culture medium then treated with graded doses of Compounds 1-5 in quadruplicates at the initiation of assay. Cytotoxicity assays are usually carried out for 96 hours after addition of test compounds. Fifty μ l of resazurin dye may be added to each well during the last 4 to 6 hours of the incubation to assess viable cells at the end of culture. Dye reduction can be determined by fluorescence spectrometry using the excitation and emission wavelengths of 535 nm and 590 nm, respectively. For analysis, the extent of resazurin reduction by the treated cells can be compared to that of the untreated control cells.

For 1 h exposure assays cells can be pulsed with the drug for 1 h and then washed; the cytotoxic effect can be determined after 96 h of incubation.

Example 13—Preparation of AC10-MC-MMAF by
Conjugation of AC10 and MC-MMAF

AC10-MC-MMAF was prepared by conjugation of AC10 and MC-MMAF following the procedure of Example 12.

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Example 17—In Vitro Cytotoxicity Data for
Selected Compounds

Table 10 shows cytotoxic effect of cAC10 Conjugates of Compounds 7-10, assayed as described in General Procedure I on a CD30+ cell line Karpas 299. Data of two separate

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experiments are presented. The cAC10 conjugates of Compounds 7 and 9 were found to be slightly more active than cAC10-val-cit-MMAE.

TABLE 10

Conjugate	IC ₅₀ (ng/mL)
cAC10-val-cit-MMAE	6
cAC10-7	1.0
cAC10-8	15
cAC10-9	0.5
cAC10-10	20

In other experiments, BR96-val-cit-MMAF was at least 250 fold more potent than the free MMAF.

General Procedure I—Cytotoxicity determination.

To evaluate the cytotoxicity of Exemplary Conjugates 7-10, cells were seeded at approximately 5-10,000 per well in 150 μ l of culture medium then treated with graded doses of Exemplary Conjugates 7-10 in quadruplicates at the initiation of assay. Cytotoxicity assays were carried out for 96 hours after addition of test compounds. Fifty μ l of the resazurin dye was added to each well during the last 4 to 6 hours of the incubation to assess viable cells at the end of culture. Dye reduction was determined by fluorescence spectrometry using the excitation and emission wavelengths of 535 nm and 590 nm, respectively. For analysis, the extent of resazurin reduction by the treated cells was compared to that of the untreated control cells.

Example 18—In Vitro Cell Proliferation Assay

Efficacy of ADC can be measured by a cell proliferation assay employing the following protocol (Promega Corp. Technical Bulletin TB288; Mendoza et al. (2002) *Cancer Res.* 62:5485-5488):

1. An aliquot of 100 μ l of cell culture containing about 10⁴ cells (SKBR-3, BT474, MCF7 or MDA-MB-468) in medium was deposited in each well of a 96-well, opaque-walled plate.
2. Control wells were prepared containing medium and without cells.
3. ADC was added to the experimental wells and incubated for 3-5 days.
4. The plates were equilibrated to room temperature for approximately 30 minutes.
5. A volume of CellTiter-Glo Reagent equal to the volume of cell culture medium present in each well was added.
6. The contents were mixed for 2 minutes on an orbital shaker to induce cell lysis.
7. The plate was incubated at room temperature for 10 minutes to stabilize the luminescence signal.
8. Luminescence was recorded and reported in graphs as RLU=relative luminescence units.

Example 19—Plasma Clearance in Rat

Plasma clearance pharmacokinetics of antibody drug conjugates and total antibody was studied in Sprague-Dawley rats (Charles River Laboratories, 250-275 gms each). Animals were dosed by bolus tail vein injection (IV Push). Approximately 300 μ l, whole blood was collected through jugular cannula, or by tail stick, into lithium/heparin anticoagulant vessels at each timepoint: 0 (predose), 10, and 30 minutes; 1, 2, 4, 8, 24 and 36 hours; and 2, 3, 4, 7, 14, 21, 28 days post dose. Total antibody was measured by

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ELISA—ECD/GxhuFc-HRP. Antibody drug conjugate was measured by ELISA—MMAE/MMAF/ECD-Bio/SA-HRP.

Example 20—Plasma Clearance in Monkey

Plasma clearance pharmacokinetics of antibody drug conjugates and total antibody can be studied in cynomolgus monkeys. FIG. 12 shows a two-stage plasma concentration clearance study after administration of H-MC-vc-MMAE to Cynomolgus monkeys at different doses: 0.5, 1.5, 2.5, and 3.0 mg/kg, administered at day 1 and day 21. Concentrations of total antibody and ADC were measured over time. (H=Trastuzumab).

Example 21—Tumor Volume In Vivo Efficacy in Transgenic Explant Mice

Animals suitable for transgenic experiments can be obtained from standard commercial sources such as Taconic (Germantown, N.Y.). Many strains are suitable, but FVB female mice are preferred because of their higher susceptibility to tumor formation. FVB males can be used for mating and vasectomized CD.1 studs can be used to stimulate pseudopregnancy. Vasectomized mice can be obtained from any commercial supplier. Founders can be bred with either FVB mice or with 129/B1.6 \times FVB p53 heterozygous mice. The mice with heterozygosity at p53 allele can be used to potentially increase tumor formation. Some F1 tumors are of mixed strain. Founder tumors can be FVB only.

Animals having tumors (allograft propagated from Fo5 mmtv transgenic mice) can be treated with a single or multiple dose by IV injection of ADC. Tumor volume can be assessed at various time points after injection.

Example 22—Synthesis of MC-MMAF Via t-Butyl Ester

MeVal-Val-Dil-Dap-Phe-OtBu (compound 1, 128.6 mg, 0.163 mmol) was suspended in CH₂Cl₂ (0.500 mL). 6-Maleimidocaproic acid (68.9 mg, 0.326 mmol) and 1,3-diisopropylcarbodiimide (0.0505 mL, 0.326 mmol) were added followed by pyridine (0.500 mL). Reaction mixture was allowed to stir for 1.0 hr. HPLC analysis indicated complete consumption of starting compound 1. Volatile organics were evaporated under reduced pressure. Product was isolated via flash column chromatography, using a step gradient from 0 to 5% Methanol in CH₂Cl₂. A total of 96 mg of pure MC-MeVal-Val-Dil-Dap-Phe-OtBu (12) (60% yield) was recovered. ES-MS m/z 981.26 [M+H]⁺; 1003.47 [M+Na]⁺; 979.65 [M-H]⁻. See FIG. 37. MC-MeVal-Val-Dil-Dap-Phe-OtBu (Compound 12, 74 mg, 0.0754 mmol) was suspended in CH₂Cl₂ (2.0 mL) and TFA (1 mL) at room temperature. After 2.5 hr, HPLC analysis indicated complete consumption of starting material. Volatile organics were evaporated under reduced pressure, and the product was isolated via preparatory RP-HPLC, using a Phenomenex C₁₂ Synergi Max-RP 80A Column (250 \times 21.20 mm). Eluent: linear gradient 10% to 90% MeCN/0.05% TFA (aq) over 30 minutes, then isocratic 90% MeCN/0.05% TFA (aq) for an additional 20 minutes. ES-MS m/z 925.33 [M+H]⁺; 947.30 [M+Na]⁺; 923.45 [M-H]⁻.

Example 23a—Synthesis of MC-MMAF (11) Via Dimethoxybenzyl Ester

Preparation of Fmoc-L-Phenylalanine-2,4-dimethoxybenzyl ester (Fmoc-Phe-ODMB) See FIG. 38.

A 3-neck, 5-L round-bottom flask was charged with Fmoc-L-Phenylalanine (200 g, 516 mmol Bachem), 2,4-dimethoxybenzyl alcohol (95.4 g, 567 mmol, Aldrich), and

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CH₂Cl₂ (2.0 L). N,N-dimethylformamide t-butyl acetal (155 mL, 586 mmol, Fluka) was added to the resulting suspension over 20 min under N₂, which resulted in a clear solution. The reaction was then stirred at room temperature overnight, after which time TLC analysis (0.42, Heptane/EtOAc=2:1) indicated that the reaction was complete. The reaction mixture was concentrated under reduced pressure to give a light yellow oil, which was redissolved in CH₂Cl₂ (200 mL) and purified through a short plug of silica gel (25 cm×25 cm, CH₂Cl₂) to give a colorless foam (250 g). MeCN (1 L) was added into the resulting foam, which totally dissolved the batch. It was then concentrated to dryness and redissolved in MeCN (1 L) and the resulting suspension was stirred for 1 h, filtered and the filter cake was rinsed with MeCN (2×200 mL) to give Fmoc-L-phenylalanine-2,4-dimethoxybenzyl ester as a white solid (113.58 g, 41%, 95.5% AUC by HPLC analysis). Data: HPLC.

Preparation L-Phenylalanine-2,4-Dimethoxybenzyl Ester (Phe-ODMB)

A 500-mL round-bottom flask was charged with Fmoc-L-phenylalanine-2,4-dimethoxybenzyl ester (26.00 g, 48.3 mmol), CH₂Cl₂ (150 mL) and diethylamine (75 mL, Acros). Mixture was stirred at room temperature and the completion monitored by HPLC. After 4 h, the mixture was concentrated (bath temp <30° C.). The residue was resuspended in CH₂Cl₂ (200 mL) and concentrated. This was repeated once. To the residue was added MeOH (20 mL), which caused the formation of a gel. This residue was diluted with CH₂Cl₂ (200 mL), concentrated and the cloudy oil left under vacuum overnight. The residue was suspended in CH₂Cl₂ (100 mL), then toluene (120 mL) was added. The mixture was concentrated and the residue left under vacuum overnight.

Data: HPLC, ¹H NMR.

Preparation of Fmoc-Dolaproine (Fmoc-Dap)

Boc-Dolaproine (58.8 g, 0.205 mol) was suspended in 4 N HCl in 1,4-dioxane (256 mL, 1.02 mol, Aldrich). After stirring for 1.5 hours, TLC analysis indicated the reaction was complete (10% MeOH/CH₂Cl₂) and the mixture was concentrated to near-dryness. Additional 1,4-dioxane was charged (50 mL) and the mixture was concentrated to dryness and dried under vacuum overnight. The resulting white solid was dissolved in H₂O (400 mL) and transferred to a 3-L, three-neck, round-bottom flask with a mechanical stirrer and temperature probe. N,N-diisopropylethylamine (214.3 mL, 1.23 mol, Acros) was added over one minute, causing an exotherm from 20.5 to 28.2° C. (internal). The mixture was cooled in an ice bath and 1,4-dioxane was added (400 mL). A solution of Fmoc-OSu (89.90 g, 0.267 mol, Advanced ChemTech) in 1,4-dioxane (400 mL) was added from an addition funnel over 15 minutes, maintaining the reaction temperature below 9° C. The mixture was allowed to warm to room temperature and stir for 19 hours, after which the mixture was concentrated by rotary evaporation to an aqueous slurry (390 g). The suspension was diluted with H₂O (750 mL) and Et₂O (750 mL), causing a copious white precipitate to form. The layers were separated, keeping the solids with the organic layer. The aqueous layer was acidified using conc. HCl (30 mL) and extracted with EtOAc (3×500 mL). The combined extracts were dried over MgSO₄, filtered and concentrated to give 59.25 g of a yellow oil A. The Et₂O extract was extracted once with sat. NaHCO₃ (200 mL), keeping the solids with the aqueous layer. The aqueous suspension was acidified using conc. HCl (50 mL) and extracted with Et₂O (50 mL) keeping the solids with the organic layer. The organic layer was filtered and concentrated to give 32.33 g of a yellow oil B. The two oils (A and B) were combined and purified by flash chromatog-

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raphy on silica gel eluting with CH₂Cl₂ (3.5 L), then 3% MeOH/CH₂Cl₂ (9 L) to give 68.23 g of Fmoc-dolaproine as a white foam (81%, 97.5% purity by HPLC (AUC)).

Preparation of Fmoc-Dap-Phe-ODMB

Crude Phe-ODMB (48.3 mmol) was suspended in anhydrous DMF (105 mL, Acros) for 5 minutes and Fmoc-Dap (19.80 g, 48.3 mmol) was added. The mixture was cooled in an ice bath and TBTU (17.08 g, 53.20 mmol, Matrix Innovations) was added. N,N-diisopropylethylamine (25.3 mL, 145.0 mmol, Acros) was added via syringe over 3 min. After 1 h, the ice bath was removed and the mixture was allowed to warm over 30 min. The mixture was poured into water (1 L) and extracted with ethyl acetate (300 mL). After separation, the aqueous layer was re-extracted with ethyl acetate (2×150 mL). The combined organic layers were washed with brine (150 mL), dried (MgSO₄) and filtered (filter paper) to remove the insolubles (inorganics and some dibenzofulvene). After concentration, the residue (41 g) was adsorbed on silica (41 g) and purified by chromatography (22 cm×8 cm column; 65% Heptane/EtOAc (2.5 L); 33% Heptane/EtOAc (3.8 L), to give 29.4 g of product as a white foam (86%, 92% purity by HPLC).

Data: HPLC, ¹H NMR, TLC (1:1 EtOAc/Heptane R_f=0.33, red in vanillin stain).

Preparation of Dap-Phe-ODMB

A 1-L round bottom flask was charged with Fmoc-Dap-Phe-ODMB (27.66 g), CH₂Cl₂ (122 mL) and diethylamine (61 mL, Acros). The solution was stirred at room temperature and the completion monitored by HPLC. After 7 h, the mixture was concentrated (bath temp. <30° C.). The residue was suspended in CH₂Cl₂ (300 mL) and concentrated. This was repeated twice. To the residue was added MeOH (20 mL) and CH₂Cl₂ (300 mL), and the solution was concentrated. The residue was suspended in CH₂Cl₂ (100 mL) and toluene (400 mL), concentrated, and the residue left under vacuum overnight to give a cream-like residue.

Data: HPLC, ¹H NMR, MS.

Preparation of Fmoc-MeVal-Val-Dil-Dap-Phe-ODMB

Crude Dap-Phe-ODMB (39.1 mmol) was suspended in anhydrous DMF (135 mL, Acros) for 5 minutes and Fmoc-MeVal-Val-Dil-OH (24.94 g, 39.1 mmol, see Example 2 for preparation) was added. The mixture was cooled in an ice bath and TBTU (13.81 g, 43.0 mmol, Matrix Innovations) was added. N,N-Diisopropylethylamine (20.5 mL, 117.3 mmol, Acros) was added via syringe over 2 minutes. After 1 hour, the ice bath was removed and the mixture was allowed to warm over 30 min. The mixture was poured into water (1.5 L) and diluted with ethyl acetate (480 mL). After standing for 15 minutes, the layers were separated and the aqueous layer was extracted with ethyl acetate (300 mL). The combined organic layers were washed with brine (200 mL), dried (MgSO₄) and filtered (filter paper) to remove insolubles (inorganics and some dibenzofulvene). After concentration, the residue (49 g) was scraped from the flask and adsorbed on silica (49 g) and purified by chromatography (15 cm×10 cm dia column; 2:1 EtOAc/Heptane (3 L), EtOAc (5 L); 250 mL fractions) to give 31.84 g of Fmoc-MeVal-Val-Dil-Dap-Phe-ODMB as a white foam (73%, 93% purity by HPLC (AUC)).

Data: HPLC, TLC (2:1 EtOAc/heptane, R_f=0.21, red in vanillin stain).

Preparation of MeVal-Val-Dil-Dap-Phe-ODMB

A 1-L, round-bottom flask was charged with Fmoc-MeVal-Val-Dil-Dap-Phe-ODMB (28.50 g), CH₂Cl₂ (80 mL) and diethylamine (40 mL). Mixture was stirred at room temperature overnight and then was concentrated under reduced pressure. The residue was adsorbed on silica (30 g)

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and purified by flash chromatography (15 cm×8 cm dia column; 2% MeOH/DCM (2 L), 3% MeOH/DCM (1 L), 6% MeOH/DCM (4 L); 250 mL fractions) to give 15.88 g of MeVal-Val-Dil-Dap-Phe-ODMB as a white foam (69%, 96% purity by HPLC (AUC)).

Data: HPLC, TLC (6% MeOH/DCM, R_f =0.24, red in vanillin stain).

Preparation of MC-MeVal-Val-Dil-Dap-Phe-ODMB

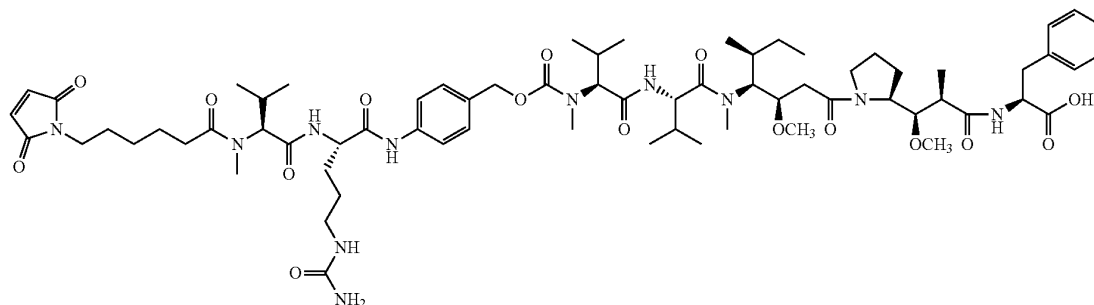
A 50-mL, round-bottom flask was charged with MeVal-Val-Dil-Dap-Phe-ODMB (750 mg, 0.85 mmol), anhydrous DMF (4 mL), maleimidocaproic acid (180 mg, 0.85 mmol), and TBTU (300 mg, 0.93 mmol, Matrix Innovations) at room temperature. N,N-Diisopropylethylamine (450 μ L, 2.57 mmol) was added via syringe. After 1.5 hours, the mixture was poured in water (50 mL) and diluted with ethyl acetate (30 mL). NaCl was added to improve the separation. After separation of the layers, the aqueous layer was extracted with ethyl acetate (25 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated. The resulting oil (1 g) was purified by flash chromatography [100 mL silica; 25% Heptane/EtOAc (100 mL), 10% Heptane/

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mm). Eluent: linear gradient 10% to 80% MeCN/0.05% TFA (aq) over 8 minutes, then isocratic 80% MeCN/0.05% TFA (aq) for an additional 12 minutes. A total of 20 mg of pure product (14) was isolated (0.02 mmol, 46% yield). ES-MS m/z 987.85 $[M+H]^+$; 1019.41 $[M+Na]^+$; 985.54 $[M-H]^-$.

MB-MeVal-Val-Dil-Dap-Phe-OtBu (Compound 14, 38 mg, 0.0385 mmol) was suspended in CH_2Cl_2 (1 mL) and TFA (1 mL). Mixture was stirred for 2.0 hr, and then volatile organics were evaporated under reduced pressure. Product was purified by preparatory RP-HPLC, using a Phenomenex C₁₂ Synergi Max-RP 80A Column (250×21.20 mm). Eluent: linear gradient 10% to 80% MeCN/0.05% TFA (aq) over 8 minutes, then isocratic 80% MeCN/0.05% TFA (aq) for an additional 12 minutes. A total of 14.4 mg of MB-MMAF product was isolated (0.015 mmol, 40% yield). ES-MS m/z 930.96 $[M+H]^+$ 952.98 $[M+Na]^+$; 929.37 $[M-H]^-$.

Example 23c—Preparation of MC-MeVal-Cit-PAB-MMAF (16)



EtOAc (200 mL), EtOAc (1.5 L)] to give MC-MeVal-Val-Dil-Dap-Phe-ODMB (13) as a white foam (521 mg, 57%, 94% purity by HPLC(AUC)).

Data: ¹H NMR, HPLC.

Preparation of MC-MeVal-Val-Dil-Dap-Phe-OH (MC-MMAF) (11)

A 50-mL, round-bottom flask was charged with MC-MeVal-Val-Dil-Dap-Phe-ODMB (Compound 13, 428 mg, 0.39 mmol) and dissolved in 2.5% TFA/ CH_2Cl_2 (20 mL). The solution turned pink-purple over 2 min. The completion was monitored by HPLC and TLC (6% MeOH/DCM, $KMnO_4$ stain). After 40 min, three drops of water were added and the cloudy pink-purple mixture was concentrated to give 521 mg of a pink residue. Purification by chromatography (15% IPA/DCM) gave 270 mg of MC-MMAF (73%, 92% purity by HPLC) as a white solid.

Example 23b—Synthesis of Analog of Mc-MMAF

MeVal-Val-Dil-Dap-Phe-OtBu (compound 1, 35 mg, 0.044 mmol) was suspended in DMF (0.250 mL). 4-(2,5-Dioxo-2,5-dihydro-pyrrol-1-yl)-benzoic acid (11 mg, 0.049 mmol) and HATU (17 mg, 0.044 mmol) were added followed by DIEA (0.031 mL, 0.17 mmol) See FIG. 39. This reaction mixture was allowed to stir for 2.0 hr. HPLC analysis indicated complete consumption of starting compound 1.

Product was isolated via preparatory RP-HPLC, using a Phenomenex C₁₂ Synergi Max-RP 80A Column (250×21.20

To a room temperature suspension of Fmoc-MeVal-OH (3.03 g, 8.57 mmol) and N,N'-disuccinimidyl carbonate (3.29 g, 12.86 mmol) in CH_2Cl_2 (80 mL) was added DIEA (4.48 mL, 25.71 mmol). This reaction mixture was allowed to stir for 3.0 hr, and then poured into a separation funnel where the organic mixture was extracted with 0.1 M HCl (aq). The crude organic residue was concentrated under reduced pressure, and the product was isolated by flash column chromatography on silica gel using a 20-100% ethyl acetate/hexanes linear gradient. A total of 2.18 g of pure Fmoc-MeVal-OSu (4.80 mmoles, 56% yield) was recovered.

To a room temperature suspension of Fmoc-MeVal-OSu (2.18 g, 4.84 mmol) in DME (13 mL) and THF (6.5 mL) was added a solution of L-citrulline (0.85 g, 4.84 mmol) and $NaHCO_3$ (0.41 g, 4.84 mmol) in H_2O (13 mL). The suspension was allowed to stir at room temperature for 16 hr, then it was extracted into tert-BuOH/ $CHCl_3/H_2O$, acidified to pH=2-3 with 1 M HCl. The organic phase was separated, dried and concentrated under reduced pressure. The residue was triturated with diethyl ether resulting in 2.01 g of Fmoc-MeVal-Cit-COOH which was used without further purification.

The crude Fmoc-MeVal-Cit-COOH was suspended in 2:1 CH_2Cl_2 /MeOH (100 mL), and to it was added p-aminobenzyl alcohol (0.97 g, 7.9 mmol) and EEDQ (1.95 g, 7.9 mmol). This suspension was allowed to stir for 125 hr, then the volatile organics were removed under reduced pressure, and the residue was purified by flash column chromatography on silica gel using a 10% MeOH/ CH_2Cl_2 . Pure Fmoc-

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MeVal-Cit-PAB-OH (0.55 g, 0.896 mmol, 18.5% yield) was recovered. ES-MS m/z 616.48 [M+H]⁺

To a suspension of Fmoc-MeVal-Cit-PAB-OH (0.55 g, 0.896 mmol) in CH₂Cl₂ (40 mL) was added STRATO-SPHERES[®] (piperazine-resin-bound) (>5 mmol/g, 150 mg). After being stirred at room temperature for 16 hr the mixture was filtered through celite (pre-washed with MeOH), and concentrated under reduced pressure. Residue was triturated with diethyl ether and hexanes. Resulting solid material, MeVal-Cit-PAB-OH, was suspended in CH₂Cl₂ (20 mL), and to it was added MC-OSu (0.28 g, 0.896 mmol), DIEA (0.17 mL, 0.99 mmol), and DMF (15 mL). This suspension was stirred for 16 hr, but HPLC analysis of the reaction mixture indicated incomplete reaction, so the suspension was concentrated under reduced pressure to a volume of 6 mL, then a 10% NaHCO₃ (aq) solution was added and the suspension stirred for an additional 16 hr.

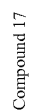
Solvent was removed under reduced pressure, and the residue was purified by flash column chromatography on silica gel using a 0-10% MeOH/CH₂Cl₂ gradient, resulting in 42 mg (0.072 mmol, 8% yield) of MC-MeVal-Cit-PAB-OH.

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To a suspension of MC-MeVal-Cit-PAB-OH (2.37 g, 4.04 mmol) and bis(nitrophenyl)carbonate (2.59 g, 8.52 mmol) in CH₂Cl₂ (10 mL) was added DIEA (1.06 mL, 6.06 mmol). This suspension was stirred for 5.5 hr, concentrated under reduced pressure and purified by trituration with diethyl ether. MC-MeVal-Cit-PAB-OCO-pNP (147 mg, 0.196 mmol) was suspended in a 1:5 pyridine/DMF solution (3 mL), and to it was added HOBt (5 mg, 0.039 mmol), DIEA (0.17 mL, 0.978 mmol) and MMAF (compound 2, 150 mg, 0.205 mmol). This reaction mixture was stirred for 16 hr at room temperature, and then purified by preparatory RP-HPLC (×3), using a Phenomenex C₁₂ Synergi Max-RP 80A Column (250×21.20 mm). Eluent: linear gradient 10% to 90% MeCN/0.05% TFA (aq) over 30 minutes, then isocratic 90% MeCN/0.05% TFA (aq) for an additional 20 minutes. MC-MeVal-Cit-PAB-MMAF (16) was obtained as a yellowish solid (24.5 mg, 0.0182, 0.45% yield). ES-MS m/z 1344.95 [M+H]⁺; 1366.94 [M+Na]⁺.

Example 23c—Preparation of Succinimide Ester of Suberyl-Val-Cit-PAB-MMAF (17)

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Compound 1 (300 mg, 0.38 mmol), Fmoc-Val-Cit-PAB-pNP (436 mg, 0.57 mmol, 1.5 eq.) were suspended in anhydrous pyridine, 5 mL. HOBt (10 mg, 0.076 mmol, 0.2 eq.) was added followed by DIEA (199 μ L, 1.14 mmol, 3 eq.). Reaction mixture was sonicated for 10 min, and then stirred overnight at room temperature. Pyridine was removed under reduced pressure, residue was re-suspended in CH_2Cl_2 . Mixture was separated by silica gel flash chromatography in a step gradient of MeOH, from 0 to 10%, in CH_2Cl_2 . Product containing fractions were pulled, concentrated, dried in vacuum overnight to give 317 mg (59% yield) of Fmoc-Val-Cit-PAB-MMAF-OtBu. ES-MS m/z 1415.8 $[\text{M}+\text{H}]^+$.

Fmoc-Val-Cit-PAB-MMAF-OtBu (100 mg) was stirred in 20% TFA/ CH_2Cl_2 (10 mL), for 2 hrs. Mixture was diluted with CH_2Cl_2 (50 mL). Organic layer was washed successively with water (2 \times 30 mL) and brine (1 \times 30 mL). Organic phase was concentrated, loaded onto pad of silica gel in 10% MeOH/ CH_2Cl_2 . Product was eluted with 30% MeOH/ CH_2Cl_2 . After drying in vacuum overnight, Fmoc-Val-Cit-PAB-MMAF was obtained as a white solid, 38 mg, 40% yield. ES-MS m/z 1357.7 $[\text{M}-\text{H}]^-$.

Fmoc-Val-Cit-PAB-MMAF, 67 mg, was suspended in CH_2Cl_2 (2 mL) diethylamine (2 mL) and DMF (2 mL). Mixture was stirred for 2 hrs at room temperature. Solvent was removed under reduced pressure. Residue was co-evaporated with pyridine (2 mL), then with toluene (2 \times 5 mL), dried in vacuum. Val-Cit-PAB-MMAF was obtained as brownish oil, and used without further purification.

All Val-Cit-PAB-MMAF prepared from 67 mg of Fmoc-Val-Cit-PAB-MMAF, was suspended in pyridine (2 mL), and added to a solution of disuccinimidyl suberate (74 mg, 0.2 mmol, 4 eq.), in pyridine (1 mL). Reaction mixture was stirred at room temperature. After 3 hrs ether (20 mL) was added. Precipitate was collected, washed with additional amount of ether. Reddish solid was suspended in 30% MeOH/ CH_2Cl_2 , filtered through a pad of silica gel with 30% MeOH/ CH_2Cl_2 as an eluent. Compound 17 was obtained as white solid, 20 mg (29% yield). ES-MS m/z 1388.5 $[\text{M}-\text{H}]^-$.

Example 24—In Vivo Efficacy of mcMMAF Antibody-Drug Conjugates

Efficacy of cAC10-mcMMAF in Karpas-299 ALCL Xenografts:

To evaluate the in vivo efficacy of cAC10-mcMMAF with an average of 4 drug moieties per antibody (cAC10-mcF4), Karpas-299 human ALCL cells were implanted subcutaneously into immunodeficient C.B-17 SCID mice (5 \times 10⁶ cells per mouse). Tumor volumes were calculated using the formula (0.5 \times L \times W²) where L and W are the longer and shorter of two bidirectional measurements. When the average tumor volume in the study animals reached approximately 100 mm³ (range 48-162) the mice were divided into 3 groups (5 mice per group) and were either left untreated or were given a single intravenous injection through the tail vein of either 1 or 2 mg/kg cAC10-mcF4 (FIG. 1). The tumors in the untreated mice grew rapidly to an average volume of >1,000 mm³ within 7 days of the start of therapy. In contrast, all of the cAC10-mcF4 treated tumor showed rapid regression with 3/5 in the 1 mg/kg group and 5/5 in the 2 mg/kg group obtaining complete tumor response. While the tumor in one of the complete responders in the 2 mg/kg group did recur approximately 4 weeks later, there were no detectable tumors in the remaining 4/5 responders in this group and in the 3 complete responders in the 1 mg/kg group at 10 weeks post therapy.

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Efficacy of cBR96-mcMMAF in L2987 NSCLC Xenografts:

cBR96 is a chimeric antibody that recognizes the Le^x antigen. To evaluate the in vivo efficacy of cBR96-mcMMAF with 4 drugs per antibody (cBR96-mcF4) L2987 non-small cell lung cancer (NSCLC) tumor fragments were implanted into athymic nude mice. When the tumors averaged approximately 100 mm³ the mice were divided into 3 groups: untreated and 2 therapy groups. For therapy, as shown in FIG. 3a, mice were administered cBR96-mcF4 at either 3 or 10 mg/kg/injection every 4 days for a total of 4 injections (q4dx4). As shown in FIG. 3b, mice were administered cBR96-mcF4 or a non-binding control conjugate, cAC10-mcF4, at 10 mg/kg/injection every 4 days for a total of 4 injections (q4dx4). As shown in FIGS. 3a and 3b, BR96-mcF4 produced pronounced tumor growth delay compared to the controls.

FIG. 2 shows an in vivo, single dose, efficacy assay of cAC10-mcMMAF in subcutaneous L540CY. For this study there were 4 mice in the untreated group and 10 in each of the treatment groups.

Example 25—In Vitro Efficacy of MC-MMAF Antibody-Drug Conjugates

Activity of cAC10-Antibody-Drug Conjugates Against CD30⁺ Cell Lines.

FIGS. 4a and 4b show dose-response curves from a representative experiment where cultures of Karpas 299 (anaplastic large cell lymphoma) and L428 (Hodgkin's Lymphoma) were incubated with serially diluted cAC10-mcMMAF (FIG. 4a) or cAC10-vcMMAF (FIG. 4b) for 96 hours. The cultures were labeled for 4 hours with 50 μ M resazurin [7-hydroxy-3H-phenoxazin-3-one 10-oxide] and the fluorescence measured. The data were reduced in GraphPad Prism version 4.00 using the 4-parameter dose-response curve fit procedure. IC₅₀ values are defined as the concentration where growth is reduced 50% compared with untreated control cultures. Each concentration was tested in quadruplicate.

Activity of cBR96-Antibody-Drug Conjugates Against Le^x Cell Lines.

FIGS. 5a and 5b show dose-response curves from a representative experiment where cultures of H3396 (breast carcinoma) and L2987 (non small cell lung carcinoma) were incubated with serially diluted cBR96-mcMMAF (FIG. 5a) or -vcMMAF (FIG. 5b) for 96 hours. The cultures were labeled for 4 hours with 50 μ M resazurin and the fluorescence measured. The data were reduced in GraphPad Prism version 4.00 using the 4-parameter dose-response curve fit procedure. IC₅₀ values are defined as the concentration where growth is reduced 50% compared with untreated control cultures. Each concentration is tested in quadruplicate.

Activity of c1F6-Antibody-Drug Conjugates Against CD70⁺ Renal Cell Carcinoma Cell Lines.

FIGS. 6a and 6b show dose-response curves from a representative experiment where cultures of Caki-1 and 786-0 cells were incubated with serially diluted c1F6-mcMMAF (FIG. 6a) or -vcMMAF (FIG. 6b) for 96 hours. The cultures were labeled for 4 hours with 50 μ M resazurin and the fluorescence measured. The data were reduced in GraphPad Prism version 4.00 using the 4-parameter dose-response curve fit procedure. IC₅₀ values are defined as the

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concentration where growth is reduced 50% compared with untreated control cultures. Each concentration is tested in quadruplicate.

Example 26—Purification of Trastuzumab

One vial containing 440 mg HERCEPTIN® (huMab4D5-8, rhuMab HER2, U.S. Pat. No. 5,821,337) antibody) was dissolved in 50 mL MES buffer (25 mM MES, 50 mM NaCl, pH 5.6) and loaded on a cation exchange column (Sephacrose S, 15 cm×1.7 cm) that had been equilibrated in the same buffer. The column was then washed with the same buffer (5 column volumes). Trastuzumab was eluted by raising the NaCl concentration of the buffer to 200 mM. Fractions containing the antibody were pooled, diluted to 10 mg/mL, and dialyzed into a buffer containing 50 mM potassium phosphate, 50 mM NaCl, 2 mM EDTA, pH 6.5.

Example 27—Preparation of Trastuzumab-MC-MMAE by Conjugation of Trastuzumab and MC-MMAE

Trastuzumab, dissolved in 500 mM sodium borate and 500 mM sodium chloride at pH 8.0 is treated with an excess of 100 mM dithiothreitol (DTT). After incubation at 37° C. for about 30 minutes, the buffer is exchanged by elution over Sephadex G25 resin and eluted with PBS with 1 mM DTPA. The thiol/Ab value is checked by determining the reduced antibody concentration from the absorbance at 280 nm of the solution and the thiol concentration by reaction with DTNB (Aldrich, Milwaukee, Wis.) and determination of the absorbance at 412 nm. The reduced antibody dissolved in PBS is chilled on ice.

The drug linker reagent, maleimidocaproyl-monomethyl auristatin E (MMAE), i.e. MC-MMAE, dissolved in DMSO, is diluted in acetonitrile and water at known concentration, and added to the chilled reduced antibody trastuzumab in PBS. After about one hour, an excess of maleimide is added to quench the reaction and cap any unreacted antibody thiol groups. The reaction mixture is concentrated by centrifugal ultrafiltration and trastuzumab-MC-MMAE is purified and desalted by elution through G25 resin in PBS, filtered through 0.2 µm filters under sterile conditions, and frozen for storage.

Example 28—Preparation of Trastuzumab-MC-MMAF by Conjugation of Trastuzumab and MC-MMAF

Trastuzumab-MC-MMAF was prepared by conjugation of trastuzumab and MC-MMAF following the procedure of Example 27.

Example 29—Preparation of Trastuzumab-MC-Val-Cit-PAB-MMAE by Conjugation of Trastuzumab and MC-Val-Cit-PAB-MMAE

Trastuzumab-MC-val-cit-PAB-MMAE was prepared by conjugation of trastuzumab and MC-val-cit-PAB-MMAE following the procedure of Example 27.

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Example 30—Preparation of Trastuzumab-MC-Val-Cit-PAB-MMAF by Conjugation of Trastuzumab and MC-Val-Cit-PAB-MMAF 9

Trastuzumab-MC-val-cit-PAB-MMAF was prepared by conjugation of trastuzumab and MC-val-cit-PAB-MMAF 9 following the procedure of Example 27.

Example 31—Rat Toxicity

The acute toxicity profile of free drugs and ADC was evaluated in adolescent Sprague-Dawley rats (75-125 gms each, Charles River Laboratories (Hollister, Calif.)). Animals were injected on day 1, complete chemistry and hematology profiles were obtained at baseline, day 3 and day 5 and a complete necropsy was performed on day 5. Liver enzyme measurements was done on all animals and routine histology as performed on three random animals for each group for the following tissues: sternum, liver, kidney, thymus, spleen, large and small intestine. The experimental groups were as follows:

Group	Administered	mg/kg	µg MMAF/ m ²	MMAF/ N/ MAB	Sex
1	Vehicle	0	0	0	2/F
2	trastuzumab-MC-val-cit-MMAF	9.94	840	4.2	6/F
3	trastuzumab-MC-val-cit-MMAF	24.90	2105	4.2	6/F
4	trastuzumab-MC(Me)-val-cit-PAB-MMAF	10.69	840	3.9	6/F
5	trastuzumab-MC(Me)-val-cit-PAB-MMAF	26.78	2105	3.9	6/F
6	trastuzumab-MC-MMAF	10.17	840	4.1	6/F
7	trastuzumab-MC-MMAF	25.50	2105	4.1	6/F
8	trastuzumab-MC-val-cit-PAB-MMAF	21.85	2105	4.8	6/F

For trastuzumab-MC-val-cit-MMAF, trastuzumab-MC(Me)-val-cit-PAB-MMAF, trastuzumab-MC-MMAF and trastuzumab-MC-val-cit-PAB-MMAF, the µg MMAF/m² was calculated using 731.5 as the MW of MMAF and 145167 as the MW of Herceptin.

The body surface area was calculated as follows: [(body weight in grams to 0.667 power)×11.8]/10000]. (Guidance for Industry and Reviewers, 2002).

The dose solutions were administered by a single intravenous bolus tail-vein injection on Study Day 1 at a dose volume of 10 mL/kg. Body weights of the animals were measured pre-dose on Study Day 1 and daily thereafter. Whole blood was collected into EDTA containing tubes for hematology analysis. Whole blood was collected into serum separator tubes for clinical chemistry analysis. Blood samples were collected pre-dose on Study Day -4, Study Day 3 and Study Day 5. Whole blood was also collected into sodium heparin containing tubes at necropsy and the plasma was frozen at -70° C. for possible later analysis. The following tissues were collected and placed in neutral buffered formalin at necropsy: liver, kidneys, heart, thymus, spleen, brain, sternum and sections of the GI tract, including stomach, large and small intestine. Sternum, small intestine, large intestine, liver, thymus, spleen and kidney were examined.

Liver associated serum enzyme levels at each timepoint were compared to a range (5th and 95th percentile) from normal female Sprague-Dawley rats. White blood cell and

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platelet counts at each timepoint were compared to a range (5th and 95th percentile) from normal female Sprague-Dawley rats.

High Dose Study in Normal Female Sprague-Dawley Rats:

Group 1:	Vehicle
Group 2:	trastuzumab-MC-MMAF, 52.24 mg/kg, 4210 $\mu\text{g}/\text{m}^2$
Group 3:	trastuzumab-MC-MMAF, 68.25 mg/kg, 5500 $\mu\text{g}/\text{m}^2$
Group 4:	trastuzumab-MC-MMAF, 86.00 mg/kg, 6930 $\mu\text{g}/\text{m}^2$

Tissues from 11 animals were submitted for routine histology. These animals had been part of an acute dose-ranging toxicity study using a trastuzumab-MC-MMAF immunoconjugate. Animals were followed for 12 days following dosing.

Example 32—Cynomolgus Monkey Toxicity/Safety

Three groups of four (2 male, 2 female) naive *Macaca fascicularis* (cynomolgus monkey) were studied for trastuzumab-MC-vc-PAB-MMAE and trastuzumab-MC-vc-PAB-MMAF. Intravenous administration was conducted at days 1 and 22 of the studies.

Sample	Group	Dose
Vehicle	1	day 1
	1M/1F	day 22
H-MC-vc-PAB-MMAE	2	180 $\mu\text{g}/\text{m}^2$ (0.5 mg/kg) at day 1
	2M/2F	1100 $\mu\text{g}/\text{m}^2$ (3.0 mg/kg) at day 22
H-MC-vc-PAB-MMAE	3	550 $\mu\text{g}/\text{m}^2$ (1.5 mg/kg) at day 8
	2M/2F	550 $\mu\text{g}/\text{m}^2$ (1.5 mg/kg) at day 29
H-MC-vc-PAB-MMAE	4	880 $\mu\text{g}/\text{m}^2$ (2.5 mg/kg) at day 15
	2M/2F	880 $\mu\text{g}/\text{m}^2$ (2.5 mg/kg) at day 36
Vehicle	1	day 1
	1M/1F	day 22
H-MC-vc-PAB-MMAF	2	180 $\mu\text{g}/\text{m}^2$ (0.5 mg/kg) at day 1
	2M/2F	1100 $\mu\text{g}/\text{m}^2$ (3.0 mg/kg) at day 22
H-MC-vc-PAB-MMAF	3	550 $\mu\text{g}/\text{m}^2$ (1.5 mg/kg) at day 1
	2M/2F	550 $\mu\text{g}/\text{m}^2$ (1.5 mg/kg) at day 22
H-MC-vc-PAB-MMAF	4	880 $\mu\text{g}/\text{m}^2$ (2.5 mg/kg) at day 1
	2M/2F	880 $\mu\text{g}/\text{m}^2$ (2.5 mg/kg) at day 22

H = trastuzumab

Dosing is expressed in surface area of an animal so as to be relevant to other species, i.e. dosage at $\mu\text{g}/\text{m}^2$ is independent of species and thus comparable between species. Formulations of ADC contained PBS, 5.4 mM sodium phosphate, 4.2 mM potassium phosphate, 140 mM sodium chloride, pH 6.5.

Blood was collected for hematology analysis predose, and at 5 min., 6 hr, 10 hr, and 1, 3, 5, 7, 14, 21 days after each dose. Erythrocyte (RBC) and platelet (PLT) counts were measured by the light scattering method. Leukocyte (WBC) count was measured by the peroxidase/basophil method. Reticulocyte count was measured by the light scattering method with cationic dye. Cell counts were measured on an Advia 120 apparatus. ALT (alanine aminotransferase) and AST (aspartate aminotransferase) were measured in U/L by UV/NADH; IFCC methodology on an Olympus AU400 apparatus, and using Total Ab ELISA—ECD/GxhuFc-HRP. Conj. Ab ELISA—MMAE/MMAFWCED-Bio/SA-HRP tests.

Example 33—Production, Characterization and Humanization of Anti-ErbB2 Monoclonal Antibody 4D5

The murine monoclonal antibody 4D5 which specifically binds the extracellular domain of ErbB2 was produced as

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described in Fendly et al. (1990) *Cancer Research* 50:1550-1558. Briefly, NIH 3T3/HER2-3₄₀₀ cells (expressing approximately 1×10^5 ErbB2 molecules/cell) produced as described in Hudziak et al. *Proc. Natl. Acad. Sci. (USA)* 84:7158-7163 (1987) were harvested with phosphate buffered saline (PBS) containing 25 mM EDTA and used to immunize BALB/c mice. The mice were given injections i.p. of 10^7 cells in 0.5 ml PBS on weeks 0, 2, 5 and 7. The mice with antisera that immunoprecipitated ^{32}P -labeled ErbB2 were given i.p. injections of a wheat germ agglutinin-Sepharose (WGA) purified ErbB2 membrane extract on weeks 9 and 13. This was followed by an i.v. injection of 0.1 ml of the ErbB2 preparation and the splenocytes were fused with mouse myeloma line X63-Ag8.653. Hybridoma supernatants were screened for ErbB2-binding by ELISA and radioimmunoprecipitation.

Epitope Mapping and Characterization

The ErbB2 epitope bound by monoclonal antibody 4D5 was determined by competitive binding analysis (Fendly et al. *Cancer Research* 50:1550-1558 (1990)). Cross-blocking studies were done by direct fluorescence on intact cells using the PANDEX™ Screen Machine to quantitate fluorescence. The monoclonal antibody was conjugated with fluorescein isothiocyanate (FITC), using established procedures (Wofsy et al. *Selected Methods in Cellular Immunology*, p. 287, Mishel and Schiigi (eds.) San Francisco: W. J. Freeman Co. (1980)). Confluent monolayers of NIH 3T3/HER2-3₄₀₀ cells were trypsinized, washed once, and resuspended at 1.75×10^6 cell/ml in cold PBS containing 0.5% bovine serum albumin (BSA) and 0.1% NaN_3 . A final concentration of 1% latex particles (IDC, Portland, Oreg.) was added to reduce clogging of the PANDEX™ plate membranes. Cells in suspension, 20 and 20 μl of purified monoclonal antibodies (100 $\mu\text{g}/\text{ml}$ to 0.1 $\mu\text{g}/\text{ml}$) were added to the PANDEX™ plate wells and incubated on ice for 30 minutes. A predetermined dilution of the FITC-labeled monoclonal antibody in 20 μl was added to each well, incubated for 30 minutes, washed, and the fluorescence was quantitated by the PANDEX™. Monoclonal antibodies were considered to share an epitope if each blocked binding of the other by 50% or greater in comparison to an irrelevant monoclonal antibody control. In this experiment, monoclonal antibody 4D5 was assigned epitope I (amino acid residues from about 529 to about 625, inclusive within the ErbB2 extracellular domain).

The growth inhibitory characteristics of monoclonal antibody 4D5 were evaluated using the breast tumor cell line, SK-BR-3 (see Hudziak et al. (1989) *Molec. Cell. Biol.* 9(3): 1165-1172). Briefly, SK-BR-3 cells were detached by using 0.25% (vol/vol) trypsin and suspended in complete medium at a density of 4×10^5 cells per ml. Aliquots of 100 μl (4×10^4 cells) were plated into 96-well microdilution plates, the cells were allowed to adhere, and 100 μl of media alone or media containing monoclonal antibody (final concentration 5 $\mu\text{g}/\text{ml}$) was then added. After 72 hours, plates were washed twice with PBS (pH 7.5), stained with crystal violet (0.5% in methanol), and analyzed for relative cell proliferation as described in Sugarman et al. (1985) *Science* 230:943-945. Monoclonal antibody 4D5 inhibited SK-BR-3 relative cell proliferation by about 56%.

Monoclonal antibody 4D5 was also evaluated for its ability to inhibit HRG-stimulated tyrosine phosphorylation of proteins in the M, 180,000 range from whole-cell lysates of MCF7 cells (Lewis et al. (1996) *Cancer Research* 56:1457-1465). MCF7 cells are reported to express all known ErbB receptors, but at relatively low levels. Since ErbB2, ErbB3, and ErbB4 have nearly identical molecular sizes, it is not possible to discern which protein is becoming

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tyrosine phosphorylated when whole-cell lysates are evaluated by Western blot analysis. However, these cells are ideal for HRG tyrosine phosphorylation assays because under the assay conditions used, in the absence of exogenously added HRG, they exhibit low to undetectable levels of tyrosine phosphorylation proteins in the M_r 180,000 range.

MCF7 cells were plated in 24-well plates and monoclonal antibodies to ErbB2 were added to each well and incubated for 30 minutes at room temperature; then rHRG β 1₁₇₇₋₂₄₄ was added to each well to a final concentration of 0.2 nM, and the incubation was continued for 8 minutes. Media was carefully aspirated from each well, and reactions were stopped by the addition of 100 μ l of SDS sample buffer (5% SDS, 25 mM DTT, and 25 mM Tris-HCl, pH 6.8). Each sample (25 μ l) was electrophoresed on a 4-12% gradient gel (Novex) and then electrophoretically transferred to polyvinylidene difluoride membrane. Antiphosphotyrosine (4G10, from UBI, used at 1 μ g/imp immunoblots were developed, and the intensity of the predominant reactive band at M_r 180,000 was quantified by reflectance densitometry, as described previously (Holmes et al. (1992) *Science* 256: 1205-1210; Sliwkowski et al. *J. Biol. Chem.* 269:14661-14665 (1994)).

Monoclonal antibody 4D5 significantly inhibited the generation of a HRG-induced tyrosine phosphorylation signal at M_r 180,000. In the absence of HRG, but was unable to stimulate tyrosine phosphorylation of proteins in the M_r 180,000 range. Also, this antibody does not cross-react with EGFR (Fendly et al. *Cancer Research* 50:1550-1558 (1990)), ErbB3, or ErbB4. Monoclonal antibody 4D5 was able to block HRG stimulation of tyrosine phosphorylation by \square 50%.

The growth inhibitory effect of monoclonal antibody 4D5 on MDA-MB-175 and SK-BR-3 cells in the presence or absence of exogenous rHRG β 1 was assessed (Schaefer et al.

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Oncogene 15:1385-1394 (1997)). ErbB2 levels in MDA-MB-175 cells are 4-6 times higher than the level found in normal breast epithelial cells and the ErbB2-ErbB4 receptor is constitutively tyrosine phosphorylated in MDA-MB-175 cells. Monoclonal antibody 4D5 was able to inhibit cell proliferation of MDA-MB-175 cells, both in the presence and absence of exogenous HRG. Inhibition of cell proliferation by 4D5 is dependent on the ErbB2 expression level (Lewis et al. *Cancer Immunol. Immunother.* 37:255-263 (1993)). A maximum inhibition of 66% in SK-BR-3 cells could be detected. However this effect could be overcome by exogenous HRG.

The murine monoclonal antibody 4D5 was humanized, using a "gene conversion mutagenesis" strategy, as described in U.S. Pat. No. 5,821,337, the entire disclosure of which is hereby expressly incorporated by reference. The humanized monoclonal antibody 4D5 used in the following experiments is designated huMAb4D5-8. This antibody is of IgG1 isotype.

REFERENCES CITED

The present invention is not to be limited in scope by the specific embodiments disclosed in the examples which are intended as illustrations of a few aspects of the invention and any embodiments that are functionally equivalent are within the scope of this invention. Indeed, various modifications of the invention in addition to those shown and described herein will become apparent to those skilled in the art and are intended to fall within the scope of the appended claims.

All references cited herein are incorporated by reference in their entirety and for all purposes to the same extent as if each individual publication or patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety for all purposes.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 35

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<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

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<223> OTHER INFORMATION: BMPR1B

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Asp Gly Glu Ser Thr Ala Pro Thr Pro Arg Pro Lys Val Leu Arg Cys
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Lys Cys His His His Cys Pro Glu Asp Ser Val Asn Asn Ile Cys Ser
35 40 45

Thr Asp Gly Tyr Cys Phe Thr Met Ile Glu Glu Asp Asp Ser Gly Leu
50 55 60

Pro Val Val Thr Ser Gly Cys Leu Gly Leu Glu Gly Ser Asp Phe Gln
65 70 75 80

Cys Arg Asp Thr Pro Ile Pro His Gln Arg Arg Ser Ile Glu Cys Cys
85 90 95

Thr Glu Arg Asn Glu Cys Asn Lys Asp Leu His Pro Thr Leu Pro Pro
100 105 110

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 Leu Ile Ser Val Thr Val Cys Ser Leu Leu Val Leu Ile Ile Leu
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 Phe Cys Tyr Phe Arg Tyr Lys Arg Gln Glu Thr Arg Pro Arg Tyr Ser
 145 150 155 160
 Ile Gly Leu Glu Gln Asp Glu Thr Tyr Ile Pro Pro Gly Glu Ser Leu
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 Arg Asp Leu Ile Glu Gln Ser Gln Ser Ser Gly Ser Gly Ser Gly Leu
 180 185 190
 Pro Leu Leu Val Gln Arg Thr Ile Ala Lys Gln Ile Gln Met Val Lys
 195 200 205
 Gln Ile Gly Lys Gly Arg Tyr Gly Glu Val Trp Met Gly Lys Trp Arg
 210 215 220
 Gly Glu Lys Val Ala Val Lys Val Phe Phe Thr Thr Glu Glu Ala Ser
 225 230 235 240
 Trp Phe Arg Glu Thr Glu Ile Tyr Gln Thr Val Leu Met Arg His Glu
 245 250 255
 Asn Ile Leu Gly Phe Ile Ala Ala Asp Ile Lys Gly Thr Gly Ser Trp
 260 265 270
 Thr Gln Leu Tyr Leu Ile Thr Asp Tyr His Glu Asn Gly Ser Leu Tyr
 275 280 285
 Asp Tyr Leu Lys Ser Thr Thr Leu Asp Ala Lys Ser Met Leu Lys Leu
 290 295 300
 Ala Tyr Ser Ser Val Ser Gly Leu Cys His Leu His Thr Glu Ile Phe
 305 310 315 320
 Ser Thr Gln Gly Lys Pro Ala Ile Ala His Arg Asp Leu Lys Ser Lys
 325 330 335
 Asn Ile Leu Val Lys Lys Asn Gly Thr Cys Cys Ile Ala Asp Leu Gly
 340 345 350
 Leu Ala Val Lys Phe Ile Ser Asp Thr Asn Glu Val Asp Ile Pro Pro
 355 360 365
 Asn Thr Arg Val Gly Thr Lys Arg Tyr Met Pro Pro Glu Val Leu Asp
 370 375 380
 Glu Ser Leu Asn Arg Asn His Phe Gln Ser Tyr Ile Met Ala Asp Met
 385 390 395 400
 Tyr Ser Phe Gly Leu Ile Leu Trp Glu Val Ala Arg Arg Cys Val Ser
 405 410 415
 Gly Gly Ile Val Glu Glu Tyr Gln Leu Pro Tyr His Asp Leu Val Pro
 420 425 430
 Ser Asp Pro Ser Tyr Glu Asp Met Arg Glu Ile Val Cys Ile Lys Lys
 435 440 445
 Leu Arg Pro Ser Phe Pro Asn Arg Trp Ser Ser Asp Glu Cys Leu Arg
 450 455 460
 Gln Met Gly Lys Leu Met Thr Glu Cys Trp Ala His Asn Pro Ala Ser
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 Ser Gln Asp Ile Lys Leu
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<210> SEQ ID NO 2
 <211> LENGTH: 507

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<213> ORGANISM: Homo sapiens
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20          25          30

Asp Gly Ser Ala Pro Ala Gly Glu Gly Glu Gly Val Thr Leu Gln Arg
35          40          45

Asn Ile Thr Leu Leu Asn Gly Val Ala Ile Ile Val Gly Thr Ile Ile
50          55          60

Gly Ser Gly Ile Phe Val Thr Pro Thr Gly Val Leu Lys Glu Ala Gly
65          70          75          80

Ser Pro Gly Leu Ala Leu Val Val Trp Ala Ala Cys Gly Val Phe Ser
85          90          95

Ile Val Gly Ala Leu Cys Tyr Ala Glu Leu Gly Thr Thr Ile Ser Lys
100         105         110

Ser Gly Gly Asp Tyr Ala Tyr Met Leu Glu Val Tyr Gly Ser Leu Pro
115         120         125

Ala Phe Leu Lys Leu Trp Ile Glu Leu Leu Ile Ile Arg Pro Ser Ser
130         135         140

Gln Tyr Ile Val Ala Leu Val Phe Ala Thr Tyr Leu Leu Lys Pro Leu
145         150         155         160

Phe Pro Thr Cys Pro Val Pro Glu Glu Ala Ala Lys Leu Val Ala Cys
165         170         175

Leu Cys Val Leu Leu Leu Thr Ala Val Asn Cys Tyr Ser Val Lys Ala
180         185         190

Ala Thr Arg Val Gln Asp Ala Phe Ala Ala Ala Lys Leu Leu Ala Leu
195         200         205

Ala Leu Ile Ile Leu Leu Gly Phe Val Gln Ile Gly Lys Gly Val Val
210         215         220

Ser Asn Leu Asp Pro Asn Phe Ser Phe Glu Gly Thr Lys Leu Asp Val
225         230         235         240

Gly Asn Ile Val Leu Ala Leu Tyr Ser Gly Leu Phe Ala Tyr Gly Gly
245         250         255

Trp Asn Tyr Leu Asn Phe Val Thr Glu Glu Met Ile Asn Pro Tyr Arg
260         265         270

Asn Leu Pro Leu Ala Ile Ile Ile Ser Leu Pro Ile Val Thr Leu Val
275         280         285

Tyr Val Leu Thr Asn Leu Ala Tyr Phe Thr Thr Leu Ser Thr Glu Gln
290         295         300

Met Leu Ser Ser Glu Ala Val Ala Val Asp Phe Gly Asn Tyr His Leu
305         310         315         320

Gly Val Met Ser Trp Ile Ile Pro Val Phe Val Gly Leu Ser Cys Phe
325         330         335

Gly Ser Val Asn Gly Ser Leu Phe Thr Ser Ser Arg Leu Phe Phe Val
340         345         350

Gly Ser Arg Glu Gly His Leu Pro Ser Ile Leu Ser Met Ile His Pro
355         360         365

Gln Leu Leu Thr Pro Val Pro Ser Leu Val Phe Thr Cys Val Met Thr
370         375         380

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Leu Leu Tyr Ala Phe Ser Lys Asp Ile Phe Ser Val Ile Asn Phe Phe
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 Ser Phe Phe Asn Trp Leu Cys Val Ala Leu Ala Ile Ile Gly Met Ile
 405 410 415
 Trp Leu Arg His Arg Lys Pro Glu Leu Glu Arg Pro Ile Lys Val Asn
 420 425 430
 Leu Ala Leu Pro Val Phe Phe Ile Leu Ala Cys Leu Phe Leu Ile Ala
 435 440 445
 Val Ser Phe Trp Lys Thr Pro Val Glu Cys Gly Ile Gly Phe Thr Ile
 450 455 460
 Ile Leu Ser Gly Leu Pro Val Tyr Phe Phe Gly Val Trp Trp Lys Asn
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 Gln Lys Leu Met Gln Val Val Pro Gln Glu Thr
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 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <223> OTHER INFORMATION: STEAP1

<400> SEQUENCE: 3

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 Gly Glu Thr Ser Met Leu Lys Arg Pro Val Leu Leu His Leu His Gln
 35 40 45
 Thr Ala His Ala Asp Glu Phe Asp Cys Pro Ser Glu Leu Gln His Thr
 50 55 60
 Gln Glu Leu Phe Pro Gln Trp His Leu Pro Ile Lys Ile Ala Ala Ile
 65 70 75 80
 Ile Ala Ser Leu Thr Phe Leu Tyr Thr Leu Leu Arg Glu Val Ile His
 85 90 95
 Pro Leu Ala Thr Ser His Gln Gln Tyr Phe Tyr Lys Ile Pro Ile Leu
 100 105 110
 Val Ile Asn Lys Val Leu Pro Met Val Ser Ile Thr Leu Leu Ala Leu
 115 120 125
 Val Tyr Leu Pro Gly Val Ile Ala Ala Ile Val Gln Leu His Asn Gly
 130 135 140
 Thr Lys Tyr Lys Lys Phe Pro His Trp Leu Asp Lys Trp Met Leu Thr
 145 150 155 160
 Arg Lys Gln Phe Gly Leu Leu Ser Phe Phe Ala Val Leu His Ala
 165 170 175
 Ile Tyr Ser Leu Ser Tyr Pro Met Arg Arg Ser Tyr Arg Tyr Lys Leu
 180 185 190
 Leu Asn Trp Ala Tyr Gln Gln Val Gln Gln Asn Lys Glu Asp Ala Trp
 195 200 205
 Ile Glu His Asp Val Trp Arg Met Glu Ile Tyr Val Ser Leu Gly Ile
 210 215 220
 Val Gly Leu Ala Ile Leu Ala Leu Leu Ala Val Thr Ser Ile Pro Ser
 225 230 235 240

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Val Ser Asp Ser Leu Thr Trp Arg Glu Phe His Tyr Ile Gln Ser Lys
245 250 255

Leu Gly Ile Val Ser Leu Leu Leu Gly Thr Ile His Ala Leu Ile Phe
260 265 270

Ala Trp Asn Lys Trp Ile Asp Ile Lys Gln Phe Val Trp Tyr Thr Pro
275 280 285

Pro Thr Phe Met Ile Ala Val Phe Leu Pro Ile Val Val Leu Ile Phe
290 295 300

Lys Ser Ile Leu Phe Leu Pro Cys Leu Arg Lys Lys Ile Leu Lys Ile
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Arg His Gly Trp Glu Asp Val Thr Lys Ile Asn Lys Thr Glu Ile Cys
325 330 335

Ser Gln Leu

<210> SEQ ID NO 4
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35 40 45

Glu Ala Met Gln Pro Ser Thr His Thr Ala Val Thr Asn Val Arg Thr
50 55 60

Ser Ile Ser Gly His Glu Ser Gln Ser Ser Val Leu Ser Asp Ser Glu
65 70 75 80

Thr Pro Lys Ala Thr Ser Pro Met Gly Thr Thr Tyr Thr Met Gly Glu
85 90 95

Thr Ser Val Ser Ile Ser Thr Ser Asp Phe Phe Glu Thr Ser Arg Ile
100 105 110

Gln Ile Glu Pro Thr Ser Ser Leu Thr Ser Gly Leu Arg Glu Thr Ser
115 120 125

Ser Ser Glu Arg Ile Ser Ser Ala Thr Glu Gly Ser Thr Val Leu Ser
130 135 140

Glu Val Pro Ser Gly Ala Thr Thr Glu Val Ser Arg Thr Glu Val Ile
145 150 155 160

Ser Ser Arg Gly Thr Ser Met Ser Gly Pro Asp Gln Phe Thr Ile Ser
165 170 175

Pro Asp Ile Ser Thr Glu Ala Ile Thr Arg Leu Ser Thr Ser Pro Ile
180 185 190

Met Thr Glu Ser Ala Glu Ser Ala Ile Thr Ile Glu Thr Gly Ser Pro
195 200 205

Gly Ala Thr Ser Glu Gly Thr Leu Thr Leu Asp Thr Ser Thr Thr Thr
210 215 220

Phe Trp Ser Gly Thr His Ser Thr Ala Ser Pro Gly Phe Ser His Ser
225 230 235 240

Glu Met Thr Thr Leu Met Ser Arg Thr Pro Gly Asp Val Pro Trp Pro
245 250 255

Ser Leu Pro Ser Val Glu Glu Ala Ser Ser Val Ser Ser Ser Leu Ser

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-continued

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Val	Lys	Thr	Thr	Asp	Met	Leu	Arg	Thr	Ser	Ser	Glu	Pro	Glu	Thr	Ser	
305					310					315					320	
Ser	Pro	Pro	Asn	Leu	Ser	Ser	Thr	Ser	Ala	Glu	Ile	Leu	Ala	Thr	Ser	
325					330					335						
Glu	Val	Thr	Lys	Asp	Arg	Glu	Lys	Ile	His	Pro	Ser	Ser	Asn	Thr	Pro	
340					345					350						
Val	Val	Asn	Val	Gly	Thr	Val	Ile	Tyr	Lys	His	Leu	Ser	Pro	Ser	Ser	
355					360					365						
Val	Leu	Ala	Asp	Leu	Val	Thr	Lys	Pro	Thr	Ser	Pro	Met	Ala	Thr		
370					375					380						
Thr	Ser	Thr	Leu	Gly	Asn	Thr	Ser	Val	Ser	Thr	Ser	Thr	Pro	Ala	Phe	
385					390					395					400	
Pro	Glu	Thr	Met	Met	Thr	Gln	Pro	Thr	Ser	Ser	Leu	Thr	Ser	Gly	Leu	
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Arg	Glu	Ile	Ser	Thr	Ser	Gln	Glu	Thr	Ser	Ser	Ala	Thr	Glu	Arg	Ser	
420					425					430						
Ala	Ser	Leu	Ser	Gly	Met	Pro	Thr	Gly	Ala	Thr	Thr	Lys	Val	Ser	Arg	
435					440					445						
Thr	Glu	Ala	Leu	Ser	Leu	Gly	Arg	Thr	Ser	Thr	Pro	Gly	Pro	Ala	Gln	
450					455					460						
Ser	Thr	Ile	Ser	Pro	Glu	Ile	Ser	Thr	Glu	Thr	Ile	Thr	Arg	Ile	Ser	
465					470					475					480	
Thr	Pro	Leu	Thr	Thr	Thr	Gly	Ser	Ala	Glu	Met	Thr	Ile	Thr	Pro	Lys	
485					490					495						
Thr	Gly	His	Ser	Gly	Ala	Ser	Ser	Gln	Gly	Thr	Phe	Thr	Leu	Asp	Thr	
500					505					510						
Ser	Ser	Arg	Ala	Ser	Trp	Pro	Gly	Thr	His	Ser	Ala	Ala	Thr	His	Arg	
515					520					525						
Ser	Pro	His	Ser	Gly	Met	Thr	Thr	Pro	Met	Ser	Arg	Gly	Pro	Glu	Asp	
530					535					540						
Val	Ser	Trp	Pro	Ser	Arg	Pro	Ser	Val	Glu	Lys	Thr	Ser	Pro	Pro	Ser	
545					550					555					560	
Ser	Leu	Val	Ser	Leu	Ser	Ala	Val	Thr	Ser	Pro	Ser	Pro	Leu	Tyr	Ser	
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Thr	Pro	Ser	Glu	Ser	Ser	His	Ser	Ser	Pro	Leu	Arg	Val	Thr	Ser	Leu	
580					585					590						
Phe	Thr	Pro	Val	Met	Met	Lys	Thr	Thr	Asp	Met	Leu	Asp	Thr	Ser	Leu	
595					600					605						
Glu	Pro	Val	Thr	Thr	Ser	Pro	Pro	Ser	Met	Asn	Ile	Thr	Ser	Asp	Glu	
610					615					620						
Ser	Leu	Ala	Thr	Ser	Lys	Ala	Thr	Met	Glu	Thr	Glu	Ala	Ile	Gln	Leu	
625					630					635					640	
Ser	Glu	Asn	Thr	Ala	Val	Thr	Gln	Met	Gly	Thr	Ile	Ser	Ala	Arg	Gln	
645					650					655						
Glu	Phe	Tyr	Ser	Ser	Tyr	Pro	Gly	Leu	Pro	Glu	Pro	Ser	Lys	Val	Thr	
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Ser	Pro	Val	Val	Thr	Ser	Ser	Thr	Ile	Lys	Asp	Ile	Val	Ser	Thr	Thr	
675					680					685						

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 1105 1110 1115 1120
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 1140 1145 1150
 Pro Leu Tyr Ser Thr Pro Ser Gly Ser Ser His Ser Ser Pro Val Pro
 1155 1160 1165
 Val Thr Ser Leu Phe Thr Ser Ile Met Met Lys Ala Thr Asp Met Leu
 1170 1175 1180
 Asp Ala Ser Leu Glu Pro Glu Thr Thr Ser Ala Pro Asn Met Asn Ile
 1185 1190 1195 1200
 Thr Ser Asp Glu Ser Leu Ala Ala Ser Lys Ala Thr Thr Glu Thr Glu
 1205 1210 1215
 Ala Ile His Val Phe Glu Asn Thr Ala Ala Ser His Val Glu Thr Thr
 1220 1225 1230
 Ser Ala Thr Glu Glu Leu Tyr Ser Ser Ser Pro Gly Phe Ser Glu Pro
 1235 1240 1245
 Thr Lys Val Ile Ser Pro Val Val Thr Ser Ser Ile Arg Asp Asn
 1250 1255 1260
 Met Val Ser Thr Thr Met Pro Gly Ser Ser Gly Ile Thr Arg Ile Glu
 1265 1270 1275 1280
 Ile Glu Ser Met Ser Ser Leu Thr Pro Gly Leu Arg Glu Thr Arg Thr
 1285 1290 1295
 Ser Gln Asp Ile Thr Ser Ser Thr Glu Thr Ser Thr Val Leu Tyr Lys
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 Ser Ser Arg Thr Ser Ile Pro Gly Pro Ala Gln Ser Thr Met Ser Leu
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 Asp Ile Ser Asp Glu Val Val Thr Arg Leu Ser Thr Ser Pro Ile Met
 1345 1350 1355 1360
 Thr Glu Ser Ala Glu Ile Thr Ile Thr Thr Gln Thr Gly Tyr Ser Leu
 1365 1370 1375
 Ala Thr Ser Gln Val Thr Leu Pro Leu Gly Thr Ser Met Thr Phe Leu
 1380 1385 1390
 Ser Gly Thr His Ser Thr Met Ser Gln Gly Leu Ser His Ser Glu Met
 1395 1400 1405
 Thr Asn Leu Met Ser Arg Gly Pro Glu Ser Leu Ser Trp Thr Ser Pro
 1410 1415 1420
 Arg Phe Val Glu Thr Thr Arg Ser Ser Ser Ser Leu Thr Ser Leu Pro
 1425 1430 1435 1440
 Leu Thr Thr Ser Leu Ser Pro Val Ser Ser Thr Leu Leu Asp Ser Ser
 1445 1450 1455
 Pro Ser Ser Pro Leu Pro Val Thr Ser Leu Ile Leu Pro Gly Leu Val
 1460 1465 1470
 Lys Thr Thr Glu Val Leu Asp Thr Ser Ser Glu Pro Lys Thr Ser Ser
 1475 1480 1485
 Ser Pro Asn Leu Ser Ser Thr Ser Val Glu Ile Pro Ala Thr Ser Glu
 1490 1495 1500
 Ile Met Thr Asp Thr Glu Lys Ile His Pro Ser Ser Asn Thr Ala Val
 1505 1510 1515 1520
 Ala Lys Val Arg Thr Ser Ser Ser Val His Glu Ser His Ser Ser Val

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Appx214

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Pro Glu Ser Arg Phe Thr Met Ser Val Thr Glu Ser Thr His His Leu
 1955 1960 1965
 Ser Thr Asp Leu Leu Pro Ser Ala Glu Thr Ile Ser Thr Gly Thr Val
 1970 1975 1980
 Met Pro Ser Leu Ser Glu Ala Met Thr Ser Phe Ala Thr Thr Gly Val
 1985 1990 1995 2000
 Pro Arg Ala Ile Ser Gly Ser Gly Ser Pro Phe Ser Arg Thr Glu Ser
 2005 2010 2015
 Gly Pro Gly Asp Ala Thr Leu Ser Thr Ile Ala Glu Ser Leu Pro Ser
 2020 2025 2030
 Ser Thr Pro Val Pro Phe Ser Ser Ser Thr Phe Thr Thr Thr Asp Ser
 2035 2040 2045
 Ser Thr Ile Pro Ala Leu His Glu Ile Thr Ser Ser Ser Ala Thr Pro
 2050 2055 2060
 Tyr Arg Val Asp Thr Ser Leu Gly Thr Glu Ser Ser Thr Thr Glu Gly
 2065 2070 2075 2080
 Arg Leu Val Met Val Ser Thr Leu Asp Thr Ser Ser Gln Pro Gly Arg
 2085 2090 2095
 Thr Ser Ser Ser Pro Ile Leu Asp Thr Arg Met Thr Glu Ser Val Glu
 2100 2105 2110
 Leu Gly Thr Val Thr Ser Ala Tyr Gln Val Pro Ser Leu Ser Thr Arg
 2115 2120 2125
 Leu Thr Arg Thr Asp Gly Ile Met Glu His Ile Thr Lys Ile Pro Asn
 2130 2135 2140
 Glu Ala Ala His Arg Gly Thr Ile Arg Pro Val Lys Gly Pro Gln Thr
 2145 2150 2155 2160
 Ser Thr Ser Pro Ala Ser Pro Lys Gly Leu His Thr Gly Gly Thr Lys
 2165 2170 2175
 Arg Met Glu Thr Thr Thr Thr Ala Leu Lys Thr Thr Thr Thr Ala Leu
 2180 2185 2190
 Lys Thr Thr Ser Arg Ala Thr Leu Thr Thr Ser Val Tyr Thr Pro Thr
 2195 2200 2205
 Leu Gly Thr Leu Thr Pro Leu Asn Ala Ser Met Gln Met Ala Ser Thr
 2210 2215 2220
 Ile Pro Thr Glu Met Met Ile Thr Thr Pro Tyr Val Phe Pro Asp Val
 2225 2230 2235 2240
 Pro Glu Thr Thr Ser Ser Leu Ala Thr Ser Leu Gly Ala Glu Thr Ser
 2245 2250 2255
 Thr Ala Leu Pro Arg Thr Thr Pro Ser Val Phe Asn Arg Glu Ser Glu
 2260 2265 2270
 Thr Thr Ala Ser Leu Val Ser Arg Ser Gly Ala Glu Arg Ser Pro Val
 2275 2280 2285
 Ile Gln Thr Leu Asp Val Ser Ser Ser Glu Pro Asp Thr Thr Ala Ser
 2290 2295 2300
 Trp Val Ile His Pro Ala Glu Thr Ile Pro Thr Val Ser Lys Thr Thr
 2305 2310 2315 2320
 Pro Asn Phe Phe His Ser Glu Leu Asp Thr Val Ser Ser Thr Ala Thr
 2325 2330 2335
 Ser His Gly Ala Asp Val Ser Ser Ala Ile Pro Thr Asn Ile Ser Pro
 2340 2345 2350
 Ser Glu Leu Asp Ala Leu Thr Pro Leu Val Thr Ile Ser Gly Thr Asp
 2355 2360 2365

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Thr Ser Thr Thr Phe Pro Thr Leu Thr Lys Ser Pro His Glu Thr Glu
 2370 2375 2380
 Thr Arg Thr Thr Trp Leu Thr His Pro Ala Glu Thr Ser Ser Thr Ile
 2385 2390 2395 2400
 Pro Arg Thr Ile Pro Asn Phe Ser His His Glu Ser Asp Ala Thr Pro
 2405 2410 2415
 Ser Ile Ala Thr Ser Pro Gly Ala Glu Thr Ser Ser Ala Ile Pro Ile
 2420 2425 2430
 Met Thr Val Ser Pro Gly Ala Glu Asp Leu Val Thr Ser Gln Val Thr
 2435 2440 2445
 Ser Ser Gly Thr Asp Arg Asn Met Thr Ile Pro Thr Leu Thr Leu Ser
 2450 2455 2460
 Pro Gly Glu Pro Lys Thr Ile Ala Ser Leu Val Thr His Pro Glu Ala
 2465 2470 2475 2480
 Gln Thr Ser Ser Ala Ile Pro Thr Ser Thr Ile Ser Pro Ala Val Ser
 2485 2490 2495
 Arg Leu Val Thr Ser Met Val Thr Ser Leu Ala Ala Lys Thr Ser Thr
 2500 2505 2510
 Thr Asn Arg Ala Leu Thr Asn Ser Pro Gly Glu Pro Ala Thr Thr Val
 2515 2520 2525
 Ser Leu Val Thr His Ser Ala Gln Thr Ser Pro Thr Val Pro Trp Thr
 2530 2535 2540
 Thr Ser Ile Phe Phe His Ser Lys Ser Asp Thr Thr Pro Ser Met Thr
 2545 2550 2555 2560
 Thr Ser His Gly Ala Glu Ser Ser Ser Ala Val Pro Thr Pro Thr Val
 2565 2570 2575
 Ser Thr Glu Val Pro Gly Val Val Thr Pro Leu Val Thr Ser Ser Arg
 2580 2585 2590
 Ala Val Ile Ser Thr Thr Ile Pro Ile Leu Thr Leu Ser Pro Gly Glu
 2595 2600 2605
 Pro Glu Thr Thr Pro Ser Met Ala Thr Ser His Gly Glu Glu Ala Ser
 2610 2615 2620
 Ser Ala Ile Pro Thr Pro Thr Val Ser Pro Gly Val Pro Gly Val Val
 2625 2630 2635 2640
 Thr Ser Leu Val Thr Ser Ser Arg Ala Val Thr Ser Thr Thr Ile Pro
 2645 2650 2655
 Ile Leu Thr Phe Ser Leu Gly Glu Pro Glu Thr Thr Pro Ser Met Ala
 2660 2665 2670
 Thr Ser His Gly Thr Glu Ala Gly Ser Ala Val Pro Thr Val Leu Pro
 2675 2680 2685
 Glu Val Pro Gly Met Val Thr Ser Leu Val Ala Ser Ser Arg Ala Val
 2690 2695 2700
 Thr Ser Thr Thr Leu Pro Thr Leu Thr Leu Ser Pro Gly Glu Pro Glu
 2705 2710 2715 2720
 Thr Thr Pro Ser Met Ala Thr Ser His Gly Ala Glu Ala Ser Ser Thr
 2725 2730 2735
 Val Pro Thr Val Ser Pro Glu Val Pro Gly Val Val Thr Ser Leu Val
 2740 2745 2750
 Thr Ser Ser Ser Gly Val Asn Ser Thr Ser Ile Pro Thr Leu Ile Leu
 2755 2760 2765
 Ser Pro Gly Glu Leu Glu Thr Thr Pro Ser Met Ala Thr Ser His Gly
 2770 2775 2780
 Ala Glu Ala Ser Ser Ala Val Pro Thr Pro Thr Val Ser Pro Gly Val

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2785	2790	2795	2800
Ser Gly Val Val Thr Pro Leu Val Thr Ser Ser Arg Ala Val Thr Ser	2805	2810	2815
Thr Thr Ile Pro Ile Leu Thr Leu Ser Ser Ser Glu Pro Glu Thr Thr	2820	2825	2830
Pro Ser Met Ala Thr Ser His Gly Val Glu Ala Ser Ser Ala Val Leu	2835	2840	2845
Thr Val Ser Pro Glu Val Pro Gly Met Val Thr Phe Leu Val Thr Ser	2850	2855	2860
Ser Arg Ala Val Thr Ser Thr Thr Ile Pro Thr Leu Thr Ile Ser Ser	2865	2870	2875
Asp Glu Pro Glu Thr Thr Thr Ser Leu Val Thr His Ser Glu Ala Lys	2885	2890	2895
Met Ile Ser Ala Ile Pro Thr Leu Gly Val Ser Pro Thr Val Gln Gly	2900	2905	2910
Leu Val Thr Ser Leu Val Thr Ser Ser Gly Ser Glu Thr Ser Ala Phe	2915	2920	2925
Ser Asn Leu Thr Val Ala Ser Ser Gln Pro Glu Thr Ile Asp Ser Trp	2930	2935	2940
Val Ala His Pro Gly Thr Glu Ala Ser Ser Val Val Pro Thr Leu Thr	2945	2950	2955
Val Ser Thr Gly Glu Pro Phe Thr Asn Ile Ser Leu Val Thr His Pro	2965	2970	2975
Ala Glu Ser Ser Ser Thr Leu Pro Arg Thr Thr Ser Arg Phe Ser His	2980	2985	2990
Ser Glu Leu Asp Thr Met Pro Ser Thr Val Thr Ser Pro Glu Ala Glu	2995	3000	3005
Ser Ser Ser Ala Ile Ser Thr Thr Thr Ile Ser Pro Gly Ile Pro Gly Val	3010	3015	3020
Leu Thr Ser Leu Val Thr Ser Ser Gly Arg Asp Ile Ser Ala Thr Phe	3025	3030	3035
Pro Thr Val Pro Glu Ser Pro His Glu Ser Glu Ala Thr Ala Ser Trp	3045	3050	3055
Val Thr His Pro Ala Val Thr Ser Thr Thr Val Pro Arg Thr Thr Pro	3060	3065	3070
Asn Tyr Ser His Ser Glu Pro Asp Thr Thr Pro Ser Ile Ala Thr Ser	3075	3080	3085
Pro Gly Ala Glu Ala Thr Ser Asp Phe Pro Thr Ile Thr Val Ser Pro	3090	3095	3100
Asp Val Pro Asp Met Val Thr Ser Gln Val Thr Ser Ser Gly Thr Asp	3105	3110	3115
Thr Ser Ile Thr Ile Pro Thr Leu Thr Leu Ser Ser Gly Glu Pro Glu	3125	3130	3135
Thr Thr Thr Ser Phe Ile Thr Tyr Ser Glu Thr His Thr Ser Ser Ala	3140	3145	3150
Ile Pro Thr Leu Pro Val Ser Pro Asp Ala Ser Lys Met Leu Thr Ser	3155	3160	3165
Leu Val Ile Ser Ser Gly Thr Asp Ser Thr Thr Thr Phe Pro Thr Leu	3170	3175	3180
Thr Glu Thr Pro Tyr Glu Pro Glu Thr Thr Ala Ile Gln Leu Ile His	3185	3190	3195
Pro Ala Glu Thr Asn Thr Met Val Pro Arg Thr Thr Pro Lys Phe Ser	3205	3210	3215

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His Ser Lys Ser Asp Thr Thr Leu Pro Val Ala Ile Thr Ser Pro Gly
 3220 3225 3230
 Pro Glu Ala Ser Ser Ala Val Ser Thr Thr Thr Ile Ser Pro Asp Met
 3235 3240 3245
 Ser Asp Leu Val Thr Ser Leu Val Pro Ser Ser Gly Thr Asp Thr Ser
 3250 3255 3260
 Thr Thr Phe Pro Thr Leu Ser Glu Thr Pro Tyr Glu Pro Glu Thr Thr
 3265 3270 3275 3280
 Ala Thr Trp Leu Thr His Pro Ala Glu Thr Ser Thr Thr Val Ser Gly
 3285 3290 3295
 Thr Ile Pro Asn Phe Ser His Arg Gly Ser Asp Thr Ala Pro Ser Met
 3300 3305 3310
 Val Thr Ser Pro Gly Val Asp Thr Arg Ser Gly Val Pro Thr Thr Thr
 3315 3320 3325
 Ile Pro Pro Ser Ile Pro Gly Val Val Thr Ser Gln Val Thr Ser Ser
 3330 3335 3340
 Ala Thr Asp Thr Ser Thr Ala Ile Pro Thr Leu Thr Pro Ser Pro Gly
 3345 3350 3355 3360
 Glu Pro Glu Thr Thr Ala Ser Ser Ala Thr His Pro Gly Thr Gln Thr
 3365 3370 3375
 Gly Phe Thr Val Pro Ile Arg Thr Val Pro Ser Ser Glu Pro Asp Thr
 3380 3385 3390
 Met Ala Ser Trp Val Thr His Pro Pro Gln Thr Ser Thr Pro Val Ser
 3395 3400 3405
 Arg Thr Thr Ser Ser Phe Ser His Ser Ser Pro Asp Ala Thr Pro Val
 3410 3415 3420
 Met Ala Thr Ser Pro Arg Thr Glu Ala Ser Ser Ala Val Leu Thr Thr
 3425 3430 3435 3440
 Ile Ser Pro Gly Ala Pro Glu Met Val Thr Ser Gln Ile Thr Ser Ser
 3445 3450 3455
 Gly Ala Ala Thr Ser Thr Thr Val Pro Thr Leu Thr His Ser Pro Gly
 3460 3465 3470
 Met Pro Glu Thr Thr Ala Leu Leu Ser Thr His Pro Arg Thr Glu Thr
 3475 3480 3485
 Ser Lys Thr Phe Pro Ala Ser Thr Val Phe Pro Gln Val Ser Glu Thr
 3490 3495 3500
 Thr Ala Ser Leu Thr Ile Arg Pro Gly Ala Glu Thr Ser Thr Ala Leu
 3505 3510 3515 3520
 Pro Thr Gln Thr Thr Ser Ser Leu Phe Thr Leu Leu Val Thr Gly Thr
 3525 3530 3535
 Ser Arg Val Asp Leu Ser Pro Thr Ala Ser Pro Gly Val Ser Ala Lys
 3540 3545 3550
 Thr Ala Pro Leu Ser Thr His Pro Gly Thr Glu Thr Ser Thr Met Ile
 3555 3560 3565
 Pro Thr Ser Thr Leu Ser Leu Gly Leu Leu Glu Thr Thr Gly Leu Leu
 3570 3575 3580
 Ala Thr Ser Ser Ser Ala Glu Thr Ser Thr Ser Thr Leu Thr Leu Thr
 3585 3590 3595 3600
 Val Ser Pro Ala Val Ser Gly Leu Ser Ser Ala Ser Ile Thr Thr Asp
 3605 3610 3615
 Lys Pro Gln Thr Val Thr Ser Trp Asn Thr Glu Thr Ser Pro Ser Val
 3620 3625 3630

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Thr Ser Val Gly Pro Pro Glu Phe Ser Arg Thr Val Thr Gly Thr Thr
 3635 3640 3645
 Met Thr Leu Ile Pro Ser Glu Met Pro Thr Pro Pro Lys Thr Ser His
 3650 3655 3660
 Gly Glu Gly Val Ser Pro Thr Thr Ile Leu Arg Thr Thr Met Val Glu
 3665 3670 3675 3680
 Ala Thr Asn Leu Ala Thr Thr Gly Ser Ser Pro Thr Val Ala Lys Thr
 3685 3690 3695
 Thr Thr Thr Phe Asn Thr Leu Ala Gly Ser Leu Phe Thr Pro Leu Thr
 3700 3705 3710
 Thr Pro Gly Met Ser Thr Leu Ala Ser Glu Ser Val Thr Ser Arg Thr
 3715 3720 3725
 Ser Tyr Asn His Arg Ser Trp Ile Ser Thr Thr Ser Ser Tyr Asn Arg
 3730 3735 3740
 Arg Tyr Trp Thr Pro Ala Thr Ser Thr Pro Val Thr Ser Thr Phe Ser
 3745 3750 3755 3760
 Pro Gly Ile Ser Thr Ser Ser Ile Pro Ser Ser Thr Ala Ala Thr Val
 3765 3770 3775
 Pro Phe Met Val Pro Phe Thr Leu Asn Phe Thr Ile Thr Asn Leu Gln
 3780 3785 3790
 Tyr Glu Glu Asp Met Arg His Pro Gly Ser Arg Lys Phe Asn Ala Thr
 3795 3800 3805
 Glu Arg Glu Leu Gln Gly Leu Leu Lys Pro Leu Phe Arg Asn Ser Ser
 3810 3815 3820
 Leu Glu Tyr Leu Tyr Ser Gly Cys Arg Leu Ala Ser Leu Arg Pro Glu
 3825 3830 3835 3840
 Lys Asp Ser Ser Ala Thr Ala Val Asp Ala Ile Cys Thr His Arg Pro
 3845 3850 3855
 Asp Pro Glu Asp Leu Gly Leu Asp Arg Glu Arg Leu Tyr Trp Glu Leu
 3860 3865 3870
 Ser Asn Leu Thr Asn Gly Ile Gln Glu Leu Gly Pro Tyr Thr Leu Asp
 3875 3880 3885
 Arg Asn Ser Leu Tyr Val Asn Gly Phe Thr His Arg Ser Ser Met Pro
 3890 3895 3900
 Thr Thr Ser Thr Pro Gly Thr Ser Thr Val Asp Val Gly Thr Ser Gly
 3905 3910 3915 3920
 Thr Pro Ser Ser Ser Pro Ser Pro Thr Thr Ala Gly Pro Leu Leu Met
 3925 3930 3935
 Pro Phe Thr Leu Asn Phe Thr Ile Thr Asn Leu Gln Tyr Glu Glu Asp
 3940 3945 3950
 Met Arg Arg Thr Gly Ser Arg Lys Phe Asn Thr Met Glu Ser Val Leu
 3955 3960 3965
 Gln Gly Leu Leu Lys Pro Leu Phe Lys Asn Thr Ser Val Gly Pro Leu
 3970 3975 3980
 Tyr Ser Gly Cys Arg Leu Thr Leu Leu Arg Pro Glu Lys Asp Gly Ala
 3985 3990 3995 4000
 Ala Thr Gly Val Asp Ala Ile Cys Thr His Arg Leu Asp Pro Lys Ser
 4005 4010 4015
 Pro Gly Leu Asn Arg Glu Gln Leu Tyr Trp Glu Leu Ser Lys Leu Thr
 4020 4025 4030
 Asn Asp Ile Glu Glu Leu Gly Pro Tyr Thr Leu Asp Arg Asn Ser Leu
 4035 4040 4045
 Tyr Val Asn Gly Phe Thr His Gln Ser Ser Val Ser Thr Thr Ser Thr

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4050	4055	4060
Pro Gly Thr Ser Thr Val Asp Leu Arg Thr Ser Gly Thr Pro Ser Ser		
4065	4070	4075 4080
Leu Ser Ser Pro Thr Ile Met Ala Ala Gly Pro Leu Leu Val Pro Phe		
	4085	4090 4095
Thr Leu Asn Phe Thr Ile Thr Asn Leu Gln Tyr Gly Glu Asp Met Gly		
	4100	4105 4110
His Pro Gly Ser Arg Lys Phe Asn Thr Thr Glu Arg Val Leu Gln Gly		
	4115	4120 4125
Leu Leu Gly Pro Ile Phe Lys Asn Thr Ser Val Gly Pro Leu Tyr Ser		
	4130	4135 4140
Gly Cys Arg Leu Thr Ser Leu Arg Ser Glu Lys Asp Gly Ala Ala Thr		
	4145	4150 4155 4160
Gly Val Asp Ala Ile Cys Ile His His Leu Asp Pro Lys Ser Pro Gly		
	4165	4170 4175
Leu Asn Arg Glu Arg Leu Tyr Trp Glu Leu Ser Gln Leu Thr Asn Gly		
	4180	4185 4190
Ile Lys Glu Leu Gly Pro Tyr Thr Leu Asp Arg Asn Ser Leu Tyr Val		
	4195	4200 4205
Asn Gly Phe Thr His Arg Thr Ser Val Pro Thr Thr Ser Thr Pro Gly		
	4210	4215 4220
Thr Ser Thr Val Asp Leu Gly Thr Ser Gly Thr Pro Phe Ser Leu Pro		
	4225	4230 4235 4240
Ser Pro Ala Thr Ala Gly Pro Leu Leu Val Leu Phe Thr Leu Asn Phe		
	4245	4250 4255
Thr Ile Thr Asn Leu Lys Tyr Glu Glu Asp Met His Arg Pro Gly Ser		
	4260	4265 4270
Arg Lys Phe Asn Thr Thr Glu Arg Val Leu Gln Thr Leu Val Gly Pro		
	4275	4280 4285
Met Phe Lys Asn Thr Ser Val Gly Leu Leu Tyr Ser Gly Cys Arg Leu		
	4290	4295 4300
Thr Leu Leu Arg Ser Glu Lys Asp Gly Ala Ala Thr Gly Val Asp Ala		
	4305	4310 4315 4320
Ile Cys Thr His Arg Leu Asp Pro Lys Ser Pro Gly Val Asp Arg Glu		
	4325	4330 4335
Gln Leu Tyr Trp Glu Leu Ser Gln Leu Thr Asn Gly Ile Lys Glu Leu		
	4340	4345 4350
Gly Pro Tyr Thr Leu Asp Arg Asn Ser Leu Tyr Val Asn Gly Phe Thr		
	4355	4360 4365
His Trp Ile Pro Val Pro Thr Ser Ser Thr Pro Gly Thr Ser Thr Val		
	4370	4375 4380
Asp Leu Gly Ser Gly Thr Pro Ser Ser Leu Pro Ser Pro Thr Ser Ala		
	4385	4390 4395 4400
Thr Ala Gly Pro Leu Leu Val Pro Phe Thr Leu Asn Phe Thr Ile Thr		
	4405	4410 4415
Asn Leu Lys Tyr Glu Glu Asp Met His Cys Pro Gly Ser Arg Lys Phe		
	4420	4425 4430
Asn Thr Thr Glu Arg Val Leu Gln Ser Leu Leu Gly Pro Met Phe Lys		
	4435	4440 4445
Asn Thr Ser Val Gly Pro Leu Tyr Ser Gly Cys Arg Leu Thr Leu Leu		
	4450	4455 4460
Arg Ser Glu Lys Asp Gly Ala Ala Thr Gly Val Asp Ala Ile Cys Thr		
	4465	4470 4475 4480

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His Arg Leu Asp Pro Lys Ser Pro Gly Val Asp Arg Glu Gln Leu Tyr
 4485 4490 4495
 Trp Glu Leu Ser Gln Leu Thr Asn Gly Ile Lys Glu Leu Gly Pro Tyr
 4500 4505 4510
 Thr Leu Asp Arg Asn Ser Leu Tyr Val Asn Gly Phe Thr His Gln Thr
 4515 4520 4525
 Ser Ala Pro Asn Thr Ser Thr Pro Gly Thr Ser Thr Val Asp Leu Gly
 4530 4535 4540
 Thr Ser Gly Thr Pro Ser Ser Leu Pro Ser Pro Thr Ser Ala Gly Pro
 4545 4550 4555 4560
 Leu Leu Val Pro Phe Thr Leu Asn Phe Thr Ile Thr Asn Leu Gln Tyr
 4565 4570 4575
 Glu Glu Asp Met His His Pro Gly Ser Arg Lys Phe Asn Thr Thr Glu
 4580 4585 4590
 Arg Val Leu Gln Gly Leu Leu Gly Pro Met Phe Lys Asn Thr Ser Val
 4595 4600 4605
 Gly Leu Leu Tyr Ser Gly Cys Arg Leu Thr Leu Leu Arg Pro Glu Lys
 4610 4615 4620
 Asn Gly Ala Ala Thr Gly Met Asp Ala Ile Cys Ser His Arg Leu Asp
 4625 4630 4635 4640
 Pro Lys Ser Pro Gly Leu Asn Arg Glu Gln Leu Tyr Trp Glu Leu Ser
 4645 4650 4655
 Gln Leu Thr His Gly Ile Lys Glu Leu Gly Pro Tyr Thr Leu Asp Arg
 4660 4665 4670
 Asn Ser Leu Tyr Val Asn Gly Phe Thr His Arg Ser Ser Val Ala Pro
 4675 4680 4685
 Thr Ser Thr Pro Gly Thr Ser Thr Val Asp Leu Gly Thr Ser Gly Thr
 4690 4695 4700
 Pro Ser Ser Leu Pro Ser Pro Thr Thr Ala Val Pro Leu Leu Val Pro
 4705 4710 4715 4720
 Phe Thr Leu Asn Phe Thr Ile Thr Asn Leu Gln Tyr Gly Glu Asp Met
 4725 4730 4735
 Arg His Pro Gly Ser Arg Lys Phe Asn Thr Thr Glu Arg Val Leu Gln
 4740 4745 4750
 Gly Leu Leu Gly Pro Leu Phe Lys Asn Ser Ser Val Gly Pro Leu Tyr
 4755 4760 4765
 Ser Gly Cys Arg Leu Ile Ser Leu Arg Ser Glu Lys Asp Gly Ala Ala
 4770 4775 4780
 Thr Gly Val Asp Ala Ile Cys Thr His His Leu Asn Pro Gln Ser Pro
 4785 4790 4795 4800
 Gly Leu Asp Arg Glu Gln Leu Tyr Trp Gln Leu Ser Gln Met Thr Asn
 4805 4810 4815
 Gly Ile Lys Glu Leu Gly Pro Tyr Thr Leu Asp Arg Asn Ser Leu Tyr
 4820 4825 4830
 Val Asn Gly Phe Thr His Arg Ser Ser Gly Leu Thr Thr Ser Thr Pro
 4835 4840 4845
 Trp Thr Ser Thr Val Asp Leu Gly Thr Ser Gly Thr Pro Ser Pro Val
 4850 4855 4860
 Pro Ser Pro Thr Thr Ala Gly Pro Leu Leu Val Pro Phe Thr Leu Asn
 4865 4870 4875 4880
 Phe Thr Ile Thr Asn Leu Gln Tyr Glu Glu Asp Met His Arg Pro Gly
 4885 4890 4895

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Ser Arg Lys Phe Asn Ala Thr Glu Arg Val Leu Gln Gly Leu Leu Ser
 4900 4905 4910
 Pro Ile Phe Lys Asn Ser Ser Val Gly Pro Leu Tyr Ser Gly Cys Arg
 4915 4920 4925
 Leu Thr Ser Leu Arg Pro Glu Lys Asp Gly Ala Ala Thr Gly Met Asp
 4930 4935 4940
 Ala Val Cys Leu Tyr His Pro Asn Pro Lys Arg Pro Gly Leu Asp Arg
 4945 4950 4955 4960
 Glu Gln Leu Tyr Trp Glu Leu Ser Gln Leu Thr His Asn Ile Thr Glu
 4965 4970 4975
 Leu Gly Pro Tyr Ser Leu Asp Arg Asp Ser Leu Tyr Val Asn Gly Phe
 4980 4985 4990
 Thr His Gln Asn Ser Val Pro Thr Thr Ser Thr Pro Gly Thr Ser Thr
 4995 5000 5005
 Val Tyr Trp Ala Thr Thr Gly Thr Pro Ser Ser Phe Pro Gly His Thr
 5010 5015 5020
 Glu Pro Gly Pro Leu Leu Ile Pro Phe Thr Phe Asn Phe Thr Ile Thr
 5025 5030 5035 5040
 Asn Leu His Tyr Glu Glu Asn Met Gln His Pro Gly Ser Arg Lys Phe
 5045 5050 5055
 Asn Thr Thr Glu Arg Val Leu Gln Gly Leu Leu Lys Pro Leu Phe Lys
 5060 5065 5070
 Asn Thr Ser Val Gly Pro Leu Tyr Ser Gly Cys Arg Leu Thr Leu Leu
 5075 5080 5085
 Arg Pro Glu Lys Gln Glu Ala Ala Thr Gly Val Asp Thr Ile Cys Thr
 5090 5095 5100
 His Arg Val Asp Pro Ile Gly Pro Gly Leu Asp Arg Glu Arg Leu Tyr
 5105 5110 5115 5120
 Trp Glu Leu Ser Gln Leu Thr Asn Ser Ile Thr Glu Leu Gly Pro Tyr
 5125 5130 5135
 Thr Leu Asp Arg Asp Ser Leu Tyr Val Asn Gly Phe Asn Pro Trp Ser
 5140 5145 5150
 Ser Val Pro Thr Thr Ser Thr Pro Gly Thr Ser Thr Val His Leu Ala
 5155 5160 5165
 Thr Ser Gly Thr Pro Ser Ser Leu Pro Gly His Thr Ala Pro Val Pro
 5170 5175 5180
 Leu Leu Ile Pro Phe Thr Leu Asn Phe Thr Ile Thr Asn Leu His Tyr
 5185 5190 5195 5200
 Glu Glu Asn Met Gln His Pro Gly Ser Arg Lys Phe Asn Thr Thr Glu
 5205 5210 5215
 Arg Val Leu Gln Gly Leu Leu Lys Pro Leu Phe Lys Ser Thr Ser Val
 5220 5225 5230
 Gly Pro Leu Tyr Ser Gly Cys Arg Leu Thr Leu Leu Arg Pro Glu Lys
 5235 5240 5245
 His Gly Ala Ala Thr Gly Val Asp Ala Ile Cys Thr Leu Arg Leu Asp
 5250 5255 5260
 Pro Thr Gly Pro Gly Leu Asp Arg Glu Arg Leu Tyr Trp Glu Leu Ser
 5265 5270 5275 5280
 Gln Leu Thr Asn Ser Val Thr Glu Leu Gly Pro Tyr Thr Leu Asp Arg
 5285 5290 5295
 Asp Ser Leu Tyr Val Asn Gly Phe Thr His Arg Ser Ser Val Pro Thr
 5300 5305 5310
 Thr Ser Ile Pro Gly Thr Ser Ala Val His Leu Glu Thr Ser Gly Thr

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5315	5320	5325
Pro Ala Ser Leu Pro Gly His Thr Ala Pro Gly Pro Leu Leu Val Pro 5330 5335 5340		
Phe Thr Leu Asn Phe Thr Ile Thr Asn Leu Gln Tyr Glu Glu Asp Met 5345 5350 5355 5360		
Arg His Pro Gly Ser Arg Lys Phe Asn Thr Thr Glu Arg Val Leu Gln 5365 5370 5375		
Gly Leu Leu Lys Pro Leu Phe Lys Ser Thr Ser Val Gly Pro Leu Tyr 5380 5385 5390		
Ser Gly Cys Arg Leu Thr Leu Leu Arg Pro Glu Lys Arg Gly Ala Ala 5395 5400 5405		
Thr Gly Val Asp Thr Ile Cys Thr His Arg Leu Asp Pro Leu Asn Pro 5410 5415 5420		
Gly Leu Asp Arg Glu Gln Leu Tyr Trp Glu Leu Ser Lys Leu Thr Arg 5425 5430 5435 5440		
Gly Ile Ile Glu Leu Gly Pro Tyr Leu Leu Asp Arg Gly Ser Leu Tyr 5445 5450 5455		
Val Asn Gly Phe Thr His Arg Asn Phe Val Pro Ile Thr Ser Thr Pro 5460 5465 5470		
Gly Thr Ser Thr Val His Leu Gly Thr Ser Glu Thr Pro Ser Ser Leu 5475 5480 5485		
Pro Arg Pro Ile Val Pro Gly Pro Leu Leu Val Pro Phe Thr Leu Asn 5490 5495 5500		
Phe Thr Ile Thr Asn Leu Gln Tyr Glu Glu Ala Met Arg His Pro Gly 5505 5510 5515 5520		
Ser Arg Lys Phe Asn Thr Thr Glu Arg Val Leu Gln Gly Leu Leu Arg 5525 5530 5535		
Pro Leu Phe Lys Asn Thr Ser Ile Gly Pro Leu Tyr Ser Ser Cys Arg 5540 5545 5550		
Leu Thr Leu Leu Arg Pro Glu Lys Asp Lys Ala Ala Thr Arg Val Asp 5555 5560 5565		
Ala Ile Cys Thr His His Pro Asp Pro Gln Ser Pro Gly Leu Asn Arg 5570 5575 5580		
Glu Gln Leu Tyr Trp Glu Leu Ser Gln Leu Thr His Gly Ile Thr Glu 5585 5590 5595 5600		
Leu Gly Pro Tyr Thr Leu Asp Arg Asp Ser Leu Tyr Val Asp Gly Phe 5605 5610 5615		
Thr His Trp Ser Pro Ile Pro Thr Thr Ser Thr Pro Gly Thr Ser Ile 5620 5625 5630		
Val Asn Leu Gly Thr Ser Gly Ile Pro Pro Ser Leu Pro Glu Thr Thr 5635 5640 5645		
Ala Thr Gly Pro Leu Leu Val Pro Phe Thr Leu Asn Phe Thr Ile Thr 5650 5655 5660		
Asn Leu Gln Tyr Glu Glu Asn Met Gly His Pro Gly Ser Arg Lys Phe 5665 5670 5675 5680		
Asn Ile Thr Glu Ser Val Leu Gln Gly Leu Leu Lys Pro Leu Phe Lys 5685 5690 5695		
Ser Thr Ser Val Gly Pro Leu Tyr Ser Gly Cys Arg Leu Thr Leu Leu 5700 5705 5710		
Arg Pro Glu Lys Asp Gly Val Ala Thr Arg Val Asp Ala Ile Cys Thr 5715 5720 5725		
His Arg Pro Asp Pro Lys Ile Pro Gly Leu Asp Arg Gln Gln Leu Tyr 5730 5735 5740		

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Trp Glu Leu Ser Gln Leu Thr His Ser Ile Thr Glu Leu Gly Pro Tyr
 5745 5750 5755 5760
 Thr Leu Asp Arg Asp Ser Leu Tyr Val Asn Gly Phe Thr Gln Arg Ser
 5765 5770 5775
 Ser Val Pro Thr Thr Ser Thr Pro Gly Thr Phe Thr Val Gln Pro Glu
 5780 5785 5790
 Thr Ser Glu Thr Pro Ser Ser Leu Pro Gly Pro Thr Ala Thr Gly Pro
 5795 5800 5805
 Val Leu Leu Pro Phe Thr Leu Asn Phe Thr Ile Ile Asn Leu Gln Tyr
 5810 5815 5820
 Glu Glu Asp Met His Arg Pro Gly Ser Arg Lys Phe Asn Thr Thr Glu
 5825 5830 5835 5840
 Arg Val Leu Gln Gly Leu Leu Met Pro Leu Phe Lys Asn Thr Ser Val
 5845 5850 5855
 Ser Ser Leu Tyr Ser Gly Cys Arg Leu Thr Leu Leu Arg Pro Glu Lys
 5860 5865 5870
 Asp Gly Ala Ala Thr Arg Val Asp Ala Val Cys Thr His Arg Pro Asp
 5875 5880 5885
 Pro Lys Ser Pro Gly Leu Asp Arg Glu Arg Leu Tyr Trp Lys Leu Ser
 5890 5895 5900
 Gln Leu Thr His Gly Ile Thr Glu Leu Gly Pro Tyr Thr Leu Asp Arg
 5905 5910 5915 5920
 His Ser Leu Tyr Val Asn Gly Phe Thr His Gln Ser Ser Met Thr Thr
 5925 5930 5935
 Thr Arg Thr Pro Asp Thr Ser Thr Met His Leu Ala Thr Ser Arg Thr
 5940 5945 5950
 Pro Ala Ser Leu Ser Gly Pro Thr Thr Ala Ser Pro Leu Leu Val Leu
 5955 5960 5965
 Phe Thr Ile Asn Phe Thr Ile Thr Asn Leu Arg Tyr Glu Glu Asn Met
 5970 5975 5980
 His His Pro Gly Ser Arg Lys Phe Asn Thr Thr Glu Arg Val Leu Gln
 5985 5990 5995 6000
 Gly Leu Leu Arg Pro Val Phe Lys Asn Thr Ser Val Gly Pro Leu Tyr
 6005 6010 6015
 Ser Gly Cys Arg Leu Thr Leu Leu Arg Pro Lys Lys Asp Gly Ala Ala
 6020 6025 6030
 Thr Lys Val Asp Ala Ile Cys Thr Tyr Arg Pro Asp Pro Lys Ser Pro
 6035 6040 6045
 Gly Leu Asp Arg Glu Gln Leu Tyr Trp Glu Leu Ser Gln Leu Thr His
 6050 6055 6060
 Ser Ile Thr Glu Leu Gly Pro Tyr Thr Leu Asp Arg Asp Ser Leu Tyr
 6065 6070 6075 6080
 Val Asn Gly Phe Thr Gln Arg Ser Ser Val Pro Thr Thr Ser Ile Pro
 6085 6090 6095
 Gly Thr Pro Thr Val Asp Leu Gly Thr Ser Gly Thr Pro Val Ser Lys
 6100 6105 6110
 Pro Gly Pro Ser Ala Ala Ser Pro Leu Leu Val Leu Phe Thr Leu Asn
 6115 6120 6125
 Phe Thr Ile Thr Asn Leu Arg Tyr Glu Glu Asn Met Gln His Pro Gly
 6130 6135 6140
 Ser Arg Lys Phe Asn Thr Thr Glu Arg Val Leu Gln Gly Leu Leu Arg
 6145 6150 6155 6160

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Ser Leu Phe Lys Ser Thr Ser Val Gly Pro Leu Tyr Ser Gly Cys Arg
 6165 6170 6175
 Leu Thr Leu Leu Arg Pro Glu Lys Asp Gly Thr Ala Thr Gly Val Asp
 6180 6185 6190
 Ala Ile Cys Thr His His Pro Asp Pro Lys Ser Pro Arg Leu Asp Arg
 6195 6200 6205
 Glu Gln Leu Tyr Trp Glu Leu Ser Gln Leu Thr His Asn Ile Thr Glu
 6210 6215 6220
 Leu Gly Pro Tyr Ala Leu Asp Asn Asp Ser Leu Phe Val Asn Gly Phe
 6225 6230 6235 6240
 Thr His Arg Ser Ser Val Ser Thr Thr Ser Thr Pro Gly Thr Pro Thr
 6245 6250 6255
 Val Tyr Leu Gly Ala Ser Lys Thr Pro Ala Ser Ile Phe Gly Pro Ser
 6260 6265 6270
 Ala Ala Ser His Leu Leu Ile Leu Phe Thr Leu Asn Phe Thr Ile Thr
 6275 6280 6285
 Asn Leu Arg Tyr Glu Glu Asn Met Trp Pro Gly Ser Arg Lys Phe Asn
 6290 6295 6300
 Thr Thr Glu Arg Val Leu Gln Gly Leu Leu Arg Pro Leu Phe Lys Asn
 6305 6310 6315 6320
 Thr Ser Val Gly Pro Leu Tyr Ser Gly Cys Arg Leu Thr Leu Leu Arg
 6325 6330 6335
 Pro Glu Lys Asp Gly Glu Ala Thr Gly Val Asp Ala Ile Cys Thr His
 6340 6345 6350
 Arg Pro Asp Pro Thr Gly Pro Gly Leu Asp Arg Glu Gln Leu Tyr Leu
 6355 6360 6365
 Glu Leu Ser Gln Leu Thr His Ser Ile Thr Glu Leu Gly Pro Tyr Thr
 6370 6375 6380
 Leu Asp Arg Asp Ser Leu Tyr Val Asn Gly Phe Thr His Arg Ser Ser
 6385 6390 6395 6400
 Val Pro Thr Thr Ser Thr Gly Val Val Ser Glu Glu Pro Phe Thr Leu
 6405 6410 6415
 Asn Phe Thr Ile Asn Asn Leu Arg Tyr Met Ala Asp Met Gly Gln Pro
 6420 6425 6430
 Gly Ser Leu Lys Phe Asn Ile Thr Asp Asn Val Met Gln His Leu Leu
 6435 6440 6445
 Ser Pro Leu Phe Gln Arg Ser Ser Leu Gly Ala Arg Tyr Thr Gly Cys
 6450 6455 6460
 Arg Val Ile Ala Leu Arg Ser Val Lys Asn Gly Ala Glu Thr Arg Val
 6465 6470 6475 6480
 Asp Leu Leu Cys Thr Tyr Leu Gln Pro Leu Ser Gly Pro Gly Leu Pro
 6485 6490 6495
 Ile Lys Gln Val Phe His Glu Leu Ser Gln Gln Thr His Gly Ile Thr
 6500 6505 6510
 Arg Leu Gly Pro Tyr Ser Leu Asp Lys Asp Ser Leu Tyr Leu Asn Gly
 6515 6520 6525
 Tyr Asn Glu Pro Gly Pro Asp Glu Pro Pro Thr Thr Pro Lys Pro Ala
 6530 6535 6540
 Thr Thr Phe Leu Pro Pro Leu Ser Glu Ala Thr Thr Ala Met Gly Tyr
 6545 6550 6555 6560
 His Leu Lys Thr Leu Thr Leu Asn Phe Thr Ile Ser Asn Leu Gln Tyr
 6565 6570 6575
 Ser Pro Asp Met Gly Lys Gly Ser Ala Thr Phe Asn Ser Thr Glu Gly

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6580	6585	6590
Val Leu Gln His Leu Leu Arg	Pro Leu Phe Gln Lys	Ser Ser Met Gly
6595	6600	6605
Pro Phe Tyr Leu Gly Cys Gln Leu Ile Ser Leu Arg	Pro Glu Lys Asp	
6610	6615	6620
Gly Ala Ala Thr Gly Val Asp Thr Thr Cys Thr Tyr His Pro Asp Pro		
6625	6630	6635 6640
Val Gly Pro Gly Leu Asp Ile Gln Gln Leu Tyr Trp Glu Leu Ser Gln		
6645	6650	6655
Leu Thr His Gly Val Thr Gln Leu Gly Phe Tyr Val Leu Asp Arg Asp		
6660	6665	6670
Ser Leu Phe Ile Asn Gly Tyr Ala Pro Gln Asn Leu Ser Ile Arg Gly		
6675	6680	6685
Glu Tyr Gln Ile Asn Phe His Ile Val Asn Trp Asn Leu Ser Asn Pro		
6690	6695	6700
Asp Pro Thr Ser Ser Glu Tyr Ile Thr Leu Leu Arg Asp Ile Gln Asp		
6705	6710	6715 6720
Lys Val Thr Thr Leu Tyr Lys Gly Ser Gln Leu His Asp Thr Phe Arg		
6725	6730	6735
Phe Cys Leu Val Thr Asn Leu Thr Met Asp Ser Val Leu Val Thr Val		
6740	6745	6750
Lys Ala Leu Phe Ser Ser Asn Leu Asp Pro Ser Leu Val Glu Gln Val		
6755	6760	6765
Phe Leu Asp Lys Thr Leu Asn Ala Ser Phe His Trp Leu Gly Ser Thr		
6770	6775	6780
Tyr Gln Leu Val Asp Ile His Val Thr Glu Met Glu Ser Ser Val Tyr		
6785	6790	6795 6800
Gln Pro Thr Ser Ser Ser Ser Thr Gln His Phe Tyr Leu Asn Phe Thr		
6805	6810	6815
Ile Thr Asn Leu Pro Tyr Ser Gln Asp Lys Ala Gln Pro Gly Thr Thr		
6820	6825	6830
Asn Tyr Gln Arg Asn Lys Arg Asn Ile Glu Asp Ala Leu Asn Gln Leu		
6835	6840	6845
Phe Arg Asn Ser Ser Ile Lys Ser Tyr Phe Ser Asp Cys Gln Val Ser		
6850	6855	6860
Thr Phe Arg Ser Val Pro Asn Arg His His Thr Gly Val Asp Ser Leu		
6865	6870	6875 6880
Cys Asn Phe Ser Pro Leu Ala Arg Arg Val Asp Arg Val Ala Ile Tyr		
6885	6890	6895
Glu Glu Phe Leu Arg Met Thr Arg Asn Gly Thr Gln Leu Gln Asn Phe		
6900	6905	6910
Thr Leu Asp Arg Ser Ser Val Leu Val Asp Gly Tyr Ser Pro Asn Arg		
6915	6920	6925
Asn Glu Pro Leu Thr Gly Asn Ser Asp Leu Pro Phe Trp Ala Val Ile		
6930	6935	6940
Leu Ile Gly Leu Ala Gly Leu Leu Gly Leu Ile Thr Cys Leu Ile Cys		
6945	6950	6955 6960
Gly Val Leu Val Thr Thr Arg Arg Arg Lys Lys Glu Gly Glu Tyr Asn		
6965	6970	6975
Val Gln Gln Gln Cys Pro Gly Tyr Tyr Gln Ser His Leu Asp Leu Glu		
6980	6985	6990
Asp Leu Gln		
6995		

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<210> SEQ ID NO 5
<211> LENGTH: 622
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: MSLN

<400> SEQUENCE: 5

Met Ala Leu Pro Thr Ala Arg Pro Leu Leu Gly Ser Cys Gly Thr Pro
1      5      10      15
Ala Leu Gly Ser Leu Leu Phe Leu Leu Phe Ser Leu Gly Trp Val Gln
20     25     30
Pro Ser Arg Thr Leu Ala Gly Glu Thr Gly Gln Glu Ala Ala Pro Leu
35     40     45
Asp Gly Val Leu Ala Asn Pro Pro Asn Ile Ser Ser Leu Ser Pro Arg
50     55     60
Gln Leu Leu Gly Phe Pro Cys Ala Glu Val Ser Gly Leu Ser Thr Glu
65     70     75     80
Arg Val Arg Glu Leu Ala Val Ala Leu Ala Gln Lys Asn Val Lys Leu
85     90     95
Ser Thr Glu Gln Leu Arg Cys Leu Ala His Arg Leu Ser Glu Pro Pro
100    105    110
Glu Asp Leu Asp Ala Leu Pro Leu Asp Leu Leu Leu Phe Leu Asn Pro
115    120    125
Asp Ala Phe Ser Gly Pro Gln Ala Cys Thr Arg Phe Phe Ser Arg Ile
130    135    140
Thr Lys Ala Asn Val Asp Leu Leu Pro Arg Gly Ala Pro Glu Arg Gln
145    150    155    160
Arg Leu Leu Pro Ala Ala Leu Ala Cys Trp Gly Val Arg Gly Ser Leu
165    170    175
Leu Ser Glu Ala Asp Val Arg Ala Leu Gly Gly Leu Ala Cys Asp Leu
180    185    190
Pro Gly Arg Phe Val Ala Glu Ser Ala Glu Val Leu Leu Pro Arg Leu
195    200    205
Val Ser Cys Pro Gly Pro Leu Asp Gln Asp Gln Gln Glu Ala Ala Arg
210    215    220
Ala Ala Leu Gln Gly Gly Gly Pro Pro Tyr Gly Pro Pro Ser Thr Trp
225    230    235    240
Ser Val Ser Thr Met Asp Ala Leu Arg Gly Leu Leu Pro Val Leu Gly
245    250    255
Gln Pro Ile Ile Arg Ser Ile Pro Gln Gly Ile Val Ala Ala Trp Arg
260    265    270
Gln Arg Ser Ser Arg Asp Pro Ser Trp Arg Gln Pro Glu Arg Thr Ile
275    280    285
Leu Arg Pro Arg Phe Arg Arg Glu Val Glu Lys Thr Ala Cys Pro Ser
290    295    300
Gly Lys Lys Ala Arg Glu Ile Asp Glu Ser Leu Ile Phe Tyr Lys Lys
305    310    315    320
Trp Glu Leu Glu Ala Cys Val Asp Ala Ala Leu Leu Ala Thr Gln Met
325    330    335
Asp Arg Val Asn Ala Ile Pro Phe Thr Tyr Glu Gln Leu Asp Val Leu
340    345    350
Lys His Lys Leu Asp Glu Leu Tyr Pro Gln Gly Tyr Pro Glu Ser Val
355    360    365

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Ile Gln His Leu Gly Tyr Leu Phe Leu Lys Met Ser Pro Glu Asp Ile
370 375 380

Arg Lys Trp Asn Val Thr Ser Leu Glu Thr Leu Lys Ala Leu Leu Glu
385 390 395 400

Val Asn Lys Gly His Glu Met Ser Pro Gln Val Ala Thr Leu Ile Asp
405 410 415

Arg Phe Val Lys Gly Arg Gly Gln Leu Asp Lys Asp Thr Leu Asp Thr
420 425 430

Leu Thr Ala Phe Tyr Pro Gly Tyr Leu Cys Ser Leu Ser Pro Glu Glu
435 440 445

Leu Ser Ser Val Pro Pro Ser Ser Ile Trp Ala Val Arg Pro Gln Asp
450 455 460

Leu Asp Thr Cys Asp Pro Arg Gln Leu Asp Val Leu Tyr Pro Lys Ala
465 470 475 480

Arg Leu Ala Phe Gln Asn Met Asn Gly Ser Glu Tyr Phe Val Lys Ile
485 490 495

Gln Ser Phe Leu Gly Gly Ala Pro Thr Glu Asp Leu Lys Ala Leu Ser
500 505 510

Gln Gln Asn Val Ser Met Asp Leu Ala Thr Phe Met Lys Leu Arg Thr
515 520 525

Asp Ala Val Leu Pro Leu Thr Val Ala Glu Val Gln Lys Leu Leu Gly
530 535 540

Pro His Val Glu Gly Leu Lys Ala Glu Glu Arg His Arg Pro Val Arg
545 550 555 560

Asp Trp Ile Leu Arg Gln Arg Gln Asp Asp Leu Asp Thr Leu Gly Leu
565 570 575

Gly Leu Gln Gly Gly Ile Pro Asn Gly Tyr Leu Val Leu Asp Leu Ser
580 585 590

Met Gln Glu Ala Leu Ser Gly Thr Pro Cys Leu Leu Gly Pro Gly Pro
595 600 605

Val Leu Thr Val Leu Ala Leu Leu Leu Ala Ser Thr Leu Ala
610 615 620

<210> SEQ ID NO 6
 <211> LENGTH: 690
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <223> OTHER INFORMATION: SLC34A2

<400> SEQUENCE: 6

Met Ala Pro Trp Pro Glu Leu Gly Asp Ala Gln Pro Asn Pro Asp Lys
1 5 10 15

Tyr Leu Glu Gly Ala Ala Gly Gln Gln Pro Thr Ala Pro Asp Lys Ser
20 25 30

Lys Glu Thr Asn Lys Thr Asp Asn Thr Glu Ala Pro Val Thr Lys Ile
35 40 45

Glu Leu Leu Pro Ser Tyr Ser Thr Ala Thr Leu Ile Asp Glu Pro Thr
50 55 60

Glu Val Asp Asp Pro Trp Asn Leu Pro Thr Leu Gln Asp Ser Gly Ile
65 70 75 80

Lys Trp Ser Glu Arg Asp Thr Lys Gly Lys Ile Leu Cys Phe Phe Gln
85 90 95

Gly Ile Gly Arg Leu Ile Leu Leu Leu Gly Phe Leu Tyr Phe Phe Val
100 105 110

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Cys Ser Leu Asp Ile Leu Ser Ser Ala Phe Gln Leu Val Gly Gly Lys
 115 120 125
 Met Ala Gly Gln Phe Phe Ser Asn Ser Ser Ile Met Ser Asn Pro Leu
 130 135 140
 Leu Gly Leu Val Ile Gly Val Leu Val Thr Val Leu Val Gln Ser Ser
 145 150 155 160
 Ser Thr Ser Thr Ser Ile Val Val Ser Met Val Ser Ser Ser Leu Leu
 165 170 175
 Thr Val Arg Ala Ala Ile Pro Ile Ile Met Gly Ala Asn Ile Gly Thr
 180 185 190
 Ser Ile Thr Asn Thr Ile Val Ala Leu Met Gln Val Gly Asp Arg Ser
 195 200 205
 Glu Phe Arg Arg Ala Phe Ala Gly Ala Thr Val His Asp Phe Phe Asn
 210 215 220
 Trp Leu Ser Val Leu Val Leu Leu Pro Val Glu Val Ala Thr His Tyr
 225 230 235 240
 Leu Glu Ile Ile Thr Gln Leu Ile Val Glu Ser Phe His Phe Lys Asn
 245 250 255
 Gly Glu Asp Ala Pro Asp Leu Leu Lys Val Ile Thr Lys Pro Phe Thr
 260 265 270
 Lys Leu Ile Val Gln Leu Asp Lys Lys Val Ile Ser Gln Ile Ala Met
 275 280 285
 Asn Asp Glu Lys Ala Lys Asn Lys Ser Leu Val Lys Ile Trp Cys Lys
 290 295 300
 Thr Phe Thr Asn Lys Thr Gln Ile Asn Val Thr Val Pro Ser Thr Ala
 305 310 315 320
 Asn Cys Thr Ser Pro Ser Leu Cys Trp Thr Asp Gly Ile Gln Asn Trp
 325 330 335
 Thr Met Lys Asn Val Thr Tyr Lys Glu Asn Ile Ala Lys Cys Gln His
 340 345 350
 Ile Phe Val Asn Phe His Leu Pro Asp Leu Ala Val Gly Thr Ile Leu
 355 360 365
 Leu Ile Leu Ser Leu Leu Val Leu Cys Gly Cys Leu Ile Met Ile Val
 370 375 380
 Lys Ile Leu Gly Ser Val Leu Lys Gly Gln Val Ala Thr Val Ile Lys
 385 390 395 400
 Lys Thr Ile Asn Thr Asp Phe Pro Phe Pro Phe Ala Trp Leu Thr Gly
 405 410 415
 Tyr Leu Ala Ile Leu Val Gly Ala Gly Met Thr Phe Ile Val Gln Ser
 420 425 430
 Ser Ser Val Phe Thr Ser Ala Leu Thr Pro Leu Ile Gly Ile Gly Val
 435 440 445
 Ile Thr Ile Glu Arg Ala Tyr Pro Leu Thr Leu Gly Ser Asn Ile Gly
 450 455 460
 Thr Thr Thr Thr Ala Ile Leu Ala Ala Leu Ala Ser Pro Gly Asn Ala
 465 470 475 480
 Leu Arg Ser Ser Leu Gln Ile Ala Leu Cys His Phe Phe Phe Asn Ile
 485 490 495
 Ser Gly Ile Leu Leu Trp Tyr Pro Ile Pro Phe Thr Arg Leu Pro Ile
 500 505 510
 Arg Met Ala Lys Gly Leu Gly Asn Ile Ser Ala Lys Tyr Arg Trp Phe
 515 520 525

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Ala Val Phe Tyr Leu Ile Ile Phe Phe Phe Leu Ile Pro Leu Thr Val
530 535 540

Phe Gly Leu Ser Leu Ala Gly Trp Arg Val Leu Val Gly Val Gly Val
545 550 555 560

Pro Val Val Phe Ile Ile Ile Leu Val Leu Cys Leu Arg Leu Leu Gln
565 570 575

Ser Arg Cys Pro Arg Val Leu Pro Lys Lys Leu Gln Asn Trp Asn Phe
580 585 590

Leu Pro Leu Trp Met Arg Ser Leu Lys Pro Trp Asp Ala Val Val Ser
595 600 605

Lys Phe Thr Gly Cys Phe Gln Met Arg Cys Cys Tyr Cys Cys Arg Val
610 615 620

Cys Cys Arg Ala Cys Cys Leu Leu Cys Gly Cys Pro Lys Cys Cys Arg
625 630 635 640

Cys Ser Lys Cys Cys Glu Asp Leu Glu Glu Ala Gln Glu Gly Gln Asp
645 650 655

Val Pro Val Lys Ala Pro Glu Thr Phe Asp Asn Ile Thr Ile Ser Arg
660 665 670

Glu Ala Gln Gly Glu Val Pro Ala Ser Asp Ser Lys Thr Glu Cys Thr
675 680 685

Ala Leu
690

<210> SEQ ID NO 7
<211> LENGTH: 1093
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: KIAA1445

<400> SEQUENCE: 7

Met Val Leu Ala Gly Pro Leu Ala Val Ser Leu Leu Leu Pro Ser Leu
1 5 10 15

Thr Leu Leu Val Ser His Leu Ser Ser Ser Gln Asp Val Ser Ser Glu
20 25 30

Pro Ser Ser Glu Gln Gln Leu Cys Ala Leu Ser Lys His Pro Thr Val
35 40 45

Ala Phe Glu Asp Leu Gln Pro Trp Val Ser Asn Phe Thr Tyr Pro Gly
50 55 60

Ala Arg Asp Phe Ser Gln Leu Ala Leu Asp Pro Ser Gly Asn Gln Leu
65 70 75 80

Ile Val Gly Ala Arg Asn Tyr Leu Phe Arg Leu Ser Leu Ala Asn Val
85 90 95

Ser Leu Leu Gln Ala Thr Glu Trp Ala Ser Ser Glu Asp Thr Arg Arg
100 105 110

Ser Cys Gln Ser Lys Gly Lys Thr Glu Glu Glu Cys Gln Asn Tyr Val
115 120 125

Arg Val Leu Ile Val Ala Gly Arg Lys Val Phe Met Cys Gly Thr Asn
130 135 140

Ala Phe Ser Pro Met Cys Thr Ser Arg Gln Val Gly Asn Leu Ser Arg
145 150 155 160

Thr Thr Glu Lys Ile Asn Gly Val Ala Arg Cys Pro Tyr Asp Pro Arg
165 170 175

His Asn Ser Thr Ala Val Ile Ser Ser Gln Gly Glu Leu Tyr Ala Ala
180 185 190

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Thr Val Ile Asp Phe Ser Gly Arg Asp Pro Ala Ile Tyr Arg Ser Leu
 195 200 205
 Gly Ser Gly Pro Pro Leu Arg Thr Ala Gln Tyr Asn Ser Lys Trp Leu
 210 215 220
 Asn Glu Pro Asn Phe Val Ala Ala Tyr Asp Ile Gly Leu Phe Ala Tyr
 225 230 235 240
 Phe Phe Leu Arg Glu Asn Ala Val Glu His Asp Cys Gly Arg Thr Val
 245 250 255
 Tyr Ser Arg Val Ala Arg Val Cys Lys Asn Asp Val Gly Gly Arg Phe
 260 265 270
 Leu Leu Glu Asp Thr Trp Thr Thr Phe Met Lys Ala Arg Leu Asn Cys
 275 280 285
 Ser Arg Pro Gly Glu Val Pro Phe Tyr Tyr Asn Glu Leu Gln Ser Ala
 290 295 300
 Phe His Leu Pro Glu Gln Asp Leu Ile Tyr Gly Val Phe Thr Thr Asn
 305 310 315 320
 Val Asn Ser Ile Ala Ala Ser Ala Val Cys Ala Phe Asn Leu Ser Ala
 325 330 335
 Ile Ser Gln Ala Phe Asn Gly Pro Phe Arg Tyr Gln Glu Asn Pro Arg
 340 345 350
 Ala Ala Trp Leu Pro Ile Ala Asn Pro Ile Pro Asn Phe Gln Cys Gly
 355 360 365
 Thr Leu Pro Glu Thr Gly Pro Asn Glu Asn Leu Thr Glu Arg Ser Leu
 370 375 380
 Gln Asp Ala Gln Arg Leu Phe Leu Met Ser Glu Ala Val Gln Pro Val
 385 390 395 400
 Thr Pro Glu Pro Cys Val Thr Gln Asp Ser Val Arg Phe Ser His Leu
 405 410 415
 Val Val Asp Leu Val Gln Ala Lys Asp Thr Leu Tyr His Val Leu Tyr
 420 425 430
 Ile Gly Thr Glu Ser Gly Thr Ile Leu Lys Ala Leu Ser Thr Ala Ser
 435 440 445
 Arg Ser Leu His Gly Cys Tyr Leu Glu Glu Leu His Val Leu Pro Pro
 450 455 460
 Gly Arg Arg Glu Pro Leu Arg Ser Leu Arg Ile Leu His Ser Ala Arg
 465 470 475 480
 Ala Leu Phe Val Gly Leu Arg Asp Gly Val Leu Arg Val Pro Leu Glu
 485 490 495
 Arg Cys Ala Ala Tyr Arg Ser Gln Gly Ala Cys Leu Gly Ala Arg Asp
 500 505 510
 Pro Tyr Cys Gly Trp Asp Gly Lys Gln Gln Arg Cys Ser Thr Leu Glu
 515 520 525
 Asp Ser Ser Asn Met Ser Leu Trp Thr Gln Asn Ile Thr Ala Cys Pro
 530 535 540
 Val Arg Asn Val Thr Arg Asp Gly Gly Phe Gly Pro Trp Ser Pro Trp
 545 550 555 560
 Gln Pro Cys Glu His Leu Asp Gly Asp Asn Ser Gly Ser Cys Leu Cys
 565 570 575
 Arg Ala Arg Ser Cys Asp Ser Pro Arg Pro Arg Cys Gly Gly Leu Asp
 580 585 590
 Cys Leu Gly Pro Ala Ile His Ile Ala Asn Cys Ser Arg Asn Gly Ala
 595 600 605
 Trp Thr Pro Trp Ser Ser Trp Ala Leu Cys Ser Thr Ser Cys Gly Ile

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610					615					620						
Gly	Phe	Gln	Val	Arg	Gln	Arg	Ser	Cys	Ser	Asn	Pro	Ala	Pro	Arg	His	
625					630					635				640		
Gly	Gly	Arg	Ile	Cys	Val	Gly	Lys	Ser	Arg	Glu	Glu	Arg	Phe	Cys	Asn	
				645					650					655		
Glu	Asn	Thr	Pro	Cys	Pro	Val	Pro	Ile	Phe	Trp	Ala	Ser	Trp	Gly	Ser	
			660					665					670			
Trp	Ser	Lys	Cys	Ser	Ser	Asn	Cys	Gly	Gly	Gly	Met	Gln	Ser	Arg	Arg	
		675					680					685				
Arg	Ala	Cys	Glu	Asn	Gly	Asn	Ser	Cys	Leu	Gly	Cys	Gly	Val	Glu	Phe	
		690					695					700				
Lys	Thr	Cys	Asn	Pro	Glu	Gly	Cys	Pro	Glu	Val	Arg	Arg	Asn	Thr	Pro	
705					710					715					720	
Trp	Thr	Pro	Trp	Leu	Pro	Val	Asn	Val	Thr	Gln	Gly	Gly	Ala	Arg	Gln	
				725					730						735	
Glu	Gln	Arg	Phe	Arg	Phe	Thr	Cys	Arg	Ala	Pro	Leu	Ala	Asp	Pro	His	
				740				745					750			
Gly	Leu	Gln	Phe	Gly	Arg	Arg	Arg	Thr	Glu	Thr	Arg	Thr	Cys	Pro	Ala	
		755						760					765			
Asp	Gly	Ser	Gly	Ser	Cys	Asp	Thr	Asp	Ala	Leu	Val	Glu	Asp	Leu	Leu	
		770				775					780					
Arg	Ser	Gly	Ser	Thr	Ser	Pro	His	Thr	Val	Ser	Gly	Gly	Trp	Ala	Ala	
785						790					795				800	
Trp	Gly	Pro	Trp	Ser	Ser	Cys	Ser	Arg	Asp	Cys	Glu	Leu	Gly	Phe	Arg	
				805					810						815	
Val	Arg	Lys	Arg	Thr	Cys	Thr	Asn	Pro	Glu	Pro	Arg	Asn	Gly	Gly	Leu	
			820					825					830			
Pro	Cys	Val	Gly	Asp	Ala	Ala	Glu	Tyr	Gln	Asp	Cys	Asn	Pro	Gln	Ala	
			835				840					845				
Cys	Pro	Val	Arg	Gly	Ala	Trp	Ser	Cys	Trp	Thr	Ser	Trp	Ser	Pro	Cys	
		850				855					860					
Ser	Ala	Ser	Cys	Gly	Gly	Gly	His	Tyr	Gln	Arg	Thr	Arg	Ser	Cys	Thr	
865				870					875						880	
Ser	Pro	Ala	Pro	Ser	Pro	Gly	Glu	Asp	Ile	Cys	Leu	Gly	Leu	His	Thr	
				885				890							895	
Glu	Glu	Ala	Leu	Cys	Ala	Thr	Gln	Ala	Cys	Pro	Glu	Gly	Trp	Ser	Pro	
			900					905					910			
Trp	Ser	Glu	Trp	Ser	Lys	Cys	Thr	Asp	Asp	Gly	Ala	Gln	Ser	Arg	Ser	
		915						920					925			
Arg	His	Cys	Glu	Glu	Leu	Leu	Pro	Gly	Ser	Ser	Ala	Cys	Ala	Gly	Asn	
			930			935					940					
Ser	Ser	Gln	Ser	Arg	Pro	Cys	Pro	Tyr	Ser	Glu	Ile	Pro	Val	Ile	Leu	
945				950					955						960	
Pro	Ala	Ser	Ser	Met	Glu	Glu	Ala	Thr	Gly	Cys	Ala	Gly	Phe	Asn	Leu	
				965					970						975	
Ile	His	Leu	Val	Ala	Thr	Gly	Ile	Ser	Cys	Phe	Leu	Gly	Ser	Gly	Leu	
			980					985							990	
Leu	Thr	Leu	Ala	Val	Tyr	Leu	Ser	Cys	Gln	His	Cys	Gln	Arg	Gln	Ser	
			995					1000					1005			
Gln	Glu	Ser	Thr	Leu	Val	His	Pro	Ala	Thr	Pro	Asn	His	Leu	His	Tyr	
			1010					1015					1020			
Lys	Gly	Gly	Gly	Thr	Pro	Lys	Asn	Glu	Lys	Tyr	Thr	Pro	Met	Glu	Phe	
1025						1030					1035				1040	

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Lys Thr Leu Asn Lys Asn Asn Leu Ile Pro Asp Asp Arg Ala Asn Phe
1045 1050 1055

Tyr Pro Leu Gln Gln Thr Asn Val Tyr Thr Thr Tyr Tyr Pro Ser
1060 1065 1070

Pro Leu Asn Lys His Ser Phe Arg Pro Glu Ala Ser Pro Gly Gln Arg
1075 1080 1085

Cys Phe Pro Asn Ser
1090

<210> SEQ ID NO 8
<211> LENGTH: 141
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: PSCA hlg

<400> SEQUENCE: 8

Met Trp Val Leu Gly Ile Ala Ala Thr Phe Cys Gly Leu Phe Leu Leu
1 5 10 15

Pro Gly Phe Ala Leu Gln Ile Gln Cys Tyr Gln Cys Glu Glu Phe Gln
20 25 30

Leu Asn Asn Asp Cys Ser Ser Pro Glu Phe Ile Val Asn Cys Thr Val
35 40 45

Asn Val Gln Asp Met Cys Gln Lys Glu Val Met Glu Gln Ser Ala Gly
50 55 60

Ile Met Tyr Arg Lys Ser Cys Ala Ser Ser Ala Cys Leu Ile Ala
65 70 75 80

Ser Ala Gly Tyr Gln Ser Phe Cys Ser Pro Gly Lys Leu Asn Ser Val
85 90 95

Cys Ile Ser Cys Cys Asn Thr Pro Leu Cys Asn Gly Pro Arg Pro Lys
100 105 110

Lys Arg Gly Ser Ser Ala Ser Ala Leu Arg Pro Gly Leu Arg Thr Thr
115 120 125

Ile Leu Phe Leu Lys Leu Ala Leu Phe Ser Ala His Cys
130 135 140

<210> SEQ ID NO 9
<211> LENGTH: 442
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: ETER

<400> SEQUENCE: 9

Met Gln Pro Pro Pro Ser Leu Cys Gly Arg Ala Leu Val Ala Leu Val
1 5 10 15

Leu Ala Cys Gly Leu Ser Arg Ile Trp Gly Glu Glu Arg Gly Phe Pro
20 25 30

Pro Asp Arg Ala Thr Pro Leu Leu Gln Thr Ala Glu Ile Met Thr Pro
35 40 45

Pro Thr Lys Thr Leu Trp Pro Lys Gly Ser Asn Ala Ser Leu Ala Arg
50 55 60

Ser Leu Ala Pro Ala Glu Val Pro Lys Gly Asp Arg Thr Ala Gly Ser
65 70 75 80

Pro Pro Arg Thr Ile Ser Pro Pro Pro Cys Gln Gly Pro Ile Glu Ile
85 90 95

Lys Glu Thr Phe Lys Tyr Ile Asn Thr Val Val Ser Cys Leu Val Phe

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100	105	110
Val Leu Gly Ile Ile Gly Asn Ser Thr Leu Leu Arg Ile Ile Tyr Lys 115 120 125		
Asn Lys Cys Met Arg Asn Gly Pro Asn Ile Leu Ile Ala Ser Leu Ala 130 135 140		
Leu Gly Asp Leu Leu His Ile Val Ile Asp Ile Pro Ile Asn Val Tyr 145 150 155 160		
Lys Leu Leu Ala Glu Asp Trp Pro Phe Gly Ala Glu Met Cys Lys Leu 165 170 175		
Val Pro Phe Ile Gln Lys Ala Ser Val Gly Ile Thr Val Leu Ser Leu 180 185 190		
Cys Ala Leu Ser Ile Asp Arg Tyr Arg Ala Val Ala Ser Trp Ser Arg 195 200 205		
Ile Lys Gly Ile Gly Val Pro Lys Trp Thr Ala Val Glu Ile Val Leu 210 215 220		
Ile Trp Val Val Ser Val Val Leu Ala Val Pro Glu Ala Ile Gly Phe 225 230 235 240		
Asp Ile Ile Thr Met Asp Tyr Lys Gly Ser Tyr Leu Arg Ile Cys Leu 245 250 255		
Leu His Pro Val Gln Lys Thr Ala Phe Met Gln Phe Tyr Lys Thr Ala 260 265 270		
Lys Asp Trp Trp Leu Phe Ser Phe Tyr Phe Cys Leu Pro Leu Ala Ile 275 280 285		
Thr Ala Phe Phe Tyr Thr Leu Met Thr Cys Glu Met Leu Arg Lys Lys 290 295 300		
Ser Gly Met Gln Ile Ala Leu Asn Asp His Leu Lys Gln Arg Arg Glu 305 310 315 320		
Val Ala Lys Thr Val Phe Cys Leu Val Leu Val Phe Ala Leu Cys Trp 325 330 335		
Leu Pro Leu His Leu Ser Arg Ile Leu Lys Leu Thr Leu Tyr Asn Gln 340 345 350		
Asn Asp Pro Asn Arg Cys Glu Leu Leu Ser Phe Leu Leu Val Leu Asp 355 360 365		
Tyr Ile Gly Ile Asn Met Ala Ser Leu Asn Ser Cys Ile Asn Pro Ile 370 375 380		
Ala Leu Tyr Leu Val Ser Lys Arg Phe Lys Asn Cys Phe Lys Ser Cys 385 390 395 400		
Leu Cys Cys Trp Cys Gln Ser Phe Glu Glu Lys Gln Ser Leu Glu Glu 405 410 415		
Lys Gln Ser Cys Leu Lys Phe Lys Ala Asn Asp His Gly Tyr Asp Asn 420 425 430		
Phe Arg Ser Ser Asn Lys Tyr Ser Ser Ser 435 440		

<210> SEQ ID NO 10
 <211> LENGTH: 783
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <223> OTHER INFORMATION: RNF124

<400> SEQUENCE: 10

Met Ser Gly Gly His Gln Leu Gln Leu Ala Ala Leu Trp Pro Trp Leu 1 5 10 15
Leu Met Ala Thr Leu Gln Ala Gly Phe Gly Arg Thr Gly Leu Val Leu

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20	25	30
Ala Ala Ala Val Glu Ser Glu Arg Ser Ala Glu Gln Lys Ala Ile Ile		
35	40	45
Arg Val Ile Pro Leu Lys Met Asp Pro Thr Gly Lys Leu Asn Leu Thr		
50	55	60
Leu Glu Gly Val Phe Ala Gly Val Ala Glu Ile Thr Pro Ala Glu Gly		
65	70	75
Lys Leu Met Gln Ser His Pro Leu Tyr Leu Cys Asn Ala Ser Asp Asp		
85	90	95
Asp Asn Leu Glu Pro Gly Phe Ile Ser Ile Val Lys Leu Glu Ser Pro		
100	105	110
Arg Arg Ala Pro Arg Pro Cys Leu Ser Leu Ala Ser Lys Ala Arg Met		
115	120	125
Ala Gly Glu Arg Gly Ala Ser Ala Val Leu Phe Asp Ile Thr Glu Asp		
130	135	140
Arg Ala Ala Ala Glu Gln Leu Gln Gln Pro Leu Gly Leu Thr Trp Pro		
145	150	155
Val Val Leu Ile Trp Gly Asn Asp Ala Glu Lys Leu Met Glu Phe Val		
165	170	175
Tyr Lys Asn Gln Lys Ala His Val Arg Ile Glu Leu Lys Glu Pro Pro		
180	185	190
Ala Trp Pro Asp Tyr Asp Val Trp Ile Leu Met Thr Val Val Gly Thr		
195	200	205
Ile Phe Val Ile Ile Leu Ala Ser Val Leu Arg Ile Arg Cys Arg Pro		
210	215	220
Arg His Ser Arg Pro Asp Pro Leu Gln Gln Arg Thr Ala Trp Ala Ile		
225	230	235
Ser Gln Leu Ala Thr Arg Arg Tyr Gln Ala Ser Cys Arg Gln Ala Arg		
245	250	255
Gly Glu Trp Pro Asp Ser Gly Ser Ser Cys Ser Ser Ala Pro Val Cys		
260	265	270
Ala Ile Cys Leu Glu Glu Phe Ser Glu Gly Gln Glu Leu Arg Val Ile		
275	280	285
Ser Cys Leu His Glu Phe His Arg Asn Cys Val Asp Pro Trp Leu His		
290	295	300
Gln His Arg Thr Cys Pro Leu Cys Val Phe Asn Ile Thr Glu Gly Asp		
305	310	315
Ser Phe Ser Gln Ser Leu Gly Pro Ser Arg Ser Tyr Gln Glu Pro Gly		
325	330	335
Arg Arg Leu His Ile Arg Gln His Pro Gly His Ala His Tyr His		
340	345	350
Leu Pro Ala Ala Tyr Leu Leu Gly Pro Ser Arg Ser Ala Val Ala Arg		
355	360	365
Pro Pro Arg Pro Gly Pro Phe Leu Pro Ser Gln Glu Pro Gly Met Gly		
370	375	380
Pro Arg His His Arg Phe Pro Arg Ala Ala His Pro Arg Ala Pro Gly		
385	390	395
Glu Gln Gln Arg Leu Ala Gly Ala Gln His Pro Tyr Ala Gln Gly Trp		
405	410	415
Gly Met Ser His Leu Gln Ser Thr Ser Gln His Pro Ala Ala Cys Pro		
420	425	430
Val Pro Leu Arg Arg Ala Arg Pro Pro Asp Ser Ser Gly Ser Gly Glu		
435	440	445

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Ser Tyr Cys Thr Glu Arg Ser Gly Tyr Leu Ala Asp Gly Pro Ala Ser
 450 455 460
 Asp Ser Ser Ser Gly Pro Cys His Gly Ser Ser Ser Asp Ser Val Val
 465 470 475 480
 Asn Cys Thr Asp Ile Ser Leu Gln Gly Val His Gly Ser Ser Ser Thr
 485 490 495
 Phe Cys Ser Ser Leu Ser Ser Asp Phe Asp Pro Leu Val Tyr Cys Ser
 500 505 510
 Pro Lys Gly Asp Pro Gln Arg Val Asp Met Gln Pro Ser Val Thr Ser
 515 520 525
 Arg Pro Arg Ser Leu Asp Ser Val Val Pro Thr Gly Glu Thr Gln Val
 530 535 540
 Ser Ser His Val His Tyr His Arg His Arg His His His Tyr Lys Lys
 545 550 555 560
 Arg Phe Gln Trp His Gly Arg Lys Pro Gly Pro Glu Thr Gly Val Pro
 565 570 575
 Gln Ser Arg Pro Pro Ile Pro Arg Thr Gln Pro Gln Pro Glu Pro Pro
 580 585 590
 Ser Pro Asp Gln Gln Val Thr Gly Ser Asn Ser Ala Ala Pro Ser Gly
 595 600 605
 Arg Leu Ser Asn Pro Gln Cys Pro Arg Ala Leu Pro Glu Pro Ala Pro
 610 615 620
 Gly Pro Val Asp Ala Ser Ser Ile Cys Pro Ser Thr Ser Ser Leu Phe
 625 630 635 640
 Asn Leu Gln Lys Ser Ser Leu Ser Ala Arg His Pro Gln Arg Lys Arg
 645 650 655
 Arg Gly Gly Pro Ser Glu Pro Thr Pro Gly Ser Arg Pro Gln Asp Ala
 660 665 670
 Thr Val His Pro Ala Cys Gln Ile Phe Pro His Tyr Thr Pro Ser Val
 675 680 685
 Ala Tyr Pro Trp Ser Pro Glu Ala His Pro Leu Ile Cys Gly Pro Pro
 690 695 700
 Gly Leu Asp Lys Arg Leu Leu Pro Glu Thr Pro Gly Pro Cys Tyr Ser
 705 710 715 720
 Asn Ser Gln Pro Val Trp Leu Cys Leu Thr Pro Arg Gln Pro Leu Glu
 725 730 735
 Pro His Pro Pro Gly Glu Gly Pro Ser Glu Trp Ser Ser Asp Thr Ala
 740 745 750
 Glu Gly Arg Pro Cys Pro Tyr Pro His Cys Gln Val Leu Ser Ala Gln
 755 760 765
 Pro Gly Ser Glu Glu Glu Leu Glu Glu Leu Cys Glu Gln Ala Val
 770 775 780

<210> SEQ ID NO 11
 <211> LENGTH: 490
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <223> OTHER INFORMATION: STEAP2

<400> SEQUENCE: 11

Met Glu Ser Ile Ser Met Met Gly Ser Pro Lys Ser Leu Ser Glu Thr
 1 5 10 15
 Val Leu Pro Asn Gly Ile Asn Gly Ile Lys Asp Ala Arg Lys Val Thr
 20 25 30

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Val Gly Val Ile Gly Ser Gly Asp Phe Ala Lys Ser Leu Thr Ile Arg
 35 40 45
 Leu Ile Arg Cys Gly Tyr His Val Val Ile Gly Ser Arg Asn Pro Lys
 50 55 60
 Phe Ala Ser Glu Phe Phe Pro His Val Val Asp Val Thr His His Glu
 65 70 75 80
 Asp Ala Leu Thr Lys Thr Asn Ile Ile Phe Val Ala Ile His Arg Glu
 85 90 95
 His Tyr Thr Ser Leu Trp Asp Leu Arg His Leu Leu Val Gly Lys Ile
 100 105 110
 Leu Ile Asp Val Ser Asn Asn Met Arg Ile Asn Gln Tyr Pro Glu Ser
 115 120 125
 Asn Ala Glu Tyr Leu Ala Ser Leu Phe Pro Asp Ser Leu Ile Val Lys
 130 135 140
 Gly Phe Asn Val Val Ser Ala Trp Ala Leu Gln Leu Gly Pro Lys Asp
 145 150 155 160
 Ala Ser Arg Gln Val Tyr Ile Cys Ser Asn Asn Ile Gln Ala Arg Gln
 165 170 175
 Gln Val Ile Glu Leu Ala Arg Gln Leu Asn Phe Ile Pro Ile Asp Leu
 180 185 190
 Gly Ser Leu Ser Ser Ala Arg Glu Ile Glu Asn Leu Pro Leu Arg Leu
 195 200 205
 Phe Thr Leu Trp Arg Gly Pro Val Val Val Ala Ile Ser Leu Ala Thr
 210 215 220
 Phe Phe Phe Leu Tyr Ser Phe Val Arg Asp Val Ile His Pro Tyr Ala
 225 230 235 240
 Arg Asn Gln Gln Ser Asp Phe Tyr Lys Ile Pro Ile Glu Ile Val Asn
 245 250 255
 Lys Thr Leu Pro Ile Val Ala Ile Thr Leu Leu Ser Leu Val Tyr Leu
 260 265 270
 Ala Gly Leu Leu Ala Ala Ala Tyr Gln Leu Tyr Tyr Gly Thr Lys Tyr
 275 280 285
 Arg Arg Phe Pro Pro Trp Leu Glu Thr Trp Leu Gln Cys Arg Lys Gln
 290 295 300
 Leu Gly Leu Leu Ser Phe Phe Phe Ala Met Val His Val Ala Tyr Ser
 305 310 315 320
 Leu Cys Leu Pro Met Arg Arg Ser Glu Arg Tyr Leu Phe Leu Asn Met
 325 330 335
 Ala Tyr Gln Gln Val His Ala Asn Ile Glu Asn Ser Trp Asn Glu Glu
 340 345 350
 Glu Val Trp Arg Ile Glu Met Tyr Ile Ser Phe Gly Ile Met Ser Leu
 355 360 365
 Gly Leu Leu Ser Leu Leu Ala Val Thr Ser Ile Pro Ser Val Ser Asn
 370 375 380
 Ala Leu Asn Trp Arg Glu Phe Ser Phe Ile Gln Ser Thr Leu Gly Tyr
 385 390 395 400
 Val Ala Leu Leu Ile Ser Thr Phe His Val Leu Ile Tyr Gly Trp Lys
 405 410 415
 Arg Ala Phe Glu Glu Glu Tyr Tyr Arg Phe Tyr Thr Pro Pro Asn Phe
 420 425 430
 Val Leu Ala Leu Val Leu Pro Ser Ile Val Ile Leu Gly Lys Ile Ile
 435 440 445

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Leu Phe Leu Pro Cys Ile Ser Gln Lys Leu Lys Arg Ile Lys Lys Gly
450 455 460

Trp Glu Lys Ser Gln Phe Leu Glu Glu Gly Ile Gly Gly Thr Ile Pro
465 470 475 480

His Val Ser Pro Glu Arg Val Thr Val Met
485 490

<210> SEQ ID NO 12
<211> LENGTH: 1214
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: TrpM4

<400> SEQUENCE: 12

Met Val Val Pro Glu Lys Glu Gln Ser Trp Ile Pro Lys Ile Phe Lys
1 5 10 15

Lys Lys Thr Cys Thr Thr Phe Ile Val Asp Ser Thr Asp Pro Gly Gly
20 25 30

Thr Leu Cys Gln Cys Gly Arg Pro Arg Thr Ala His Pro Ala Val Ala
35 40 45

Met Glu Asp Ala Phe Gly Ala Ala Val Val Thr Val Trp Asp Ser Asp
50 55 60

Ala His Thr Thr Glu Lys Pro Thr Asp Ala Tyr Gly Glu Leu Asp Phe
65 70 75 80

Thr Gly Ala Gly Arg Lys His Ser Asn Phe Leu Arg Leu Ser Asp Arg
85 90 95

Thr Asp Pro Ala Ala Val Tyr Ser Leu Val Thr Arg Thr Trp Gly Phe
100 105 110

Arg Ala Pro Asn Leu Val Val Ser Val Leu Gly Gly Ser Gly Gly Pro
115 120 125

Val Leu Gln Thr Trp Leu Gln Asp Leu Leu Arg Arg Gly Leu Val Arg
130 135 140

Ala Ala Gln Ser Thr Gly Ala Trp Ile Val Thr Gly Gly Leu His Thr
145 150 155 160

Gly Ile Gly Arg His Val Gly Val Ala Val Arg Asp His Gln Met Ala
165 170 175

Ser Thr Gly Gly Thr Lys Val Val Ala Met Gly Val Ala Pro Trp Gly
180 185 190

Val Val Arg Asn Arg Asp Thr Leu Ile Asn Pro Lys Gly Ser Phe Pro
195 200 205

Ala Arg Tyr Arg Trp Arg Gly Asp Pro Glu Asp Gly Val Gln Phe Pro
210 215 220

Leu Asp Tyr Asn Tyr Ser Ala Phe Phe Leu Val Asp Asp Gly Thr His
225 230 235 240

Gly Cys Leu Gly Gly Glu Asn Arg Phe Arg Leu Arg Leu Glu Ser Tyr
245 250 255

Ile Ser Gln Gln Lys Thr Gly Val Gly Gly Thr Gly Ile Asp Ile Pro
260 265 270

Val Leu Leu Leu Ile Asp Gly Asp Glu Lys Met Leu Thr Arg Ile
275 280 285

Glu Asn Ala Thr Gln Ala Gln Leu Pro Cys Leu Leu Val Ala Gly Ser
290 295 300

Gly Gly Ala Ala Asp Cys Leu Ala Glu Thr Leu Glu Asp Thr Leu Ala
305 310 315 320

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740					745					750						
Ser	Gly	Arg	Pro	Gly	Cys	Cys	Gly	Gly	Arg	Cys	Gly	Gly	Arg	Arg	Cys	
755					760					765						
Leu	Arg	Arg	Trp	Phe	His	Phe	Trp	Gly	Ala	Pro	Val	Thr	Ile	Phe	Met	
770					775					780						
Gly	Asn	Val	Val	Ser	Tyr	Leu	Leu	Phe	Leu	Leu	Leu	Phe	Ser	Arg	Val	
785					790					795					800	
Leu	Leu	Val	Asp	Phe	Gln	Pro	Ala	Pro	Pro	Gly	Ser	Leu	Glu	Leu	Leu	
805					810					815						
Leu	Tyr	Phe	Trp	Ala	Phe	Thr	Leu	Leu	Cys	Glu	Glu	Leu	Arg	Gln	Gly	
820					825					830						
Leu	Ser	Gly	Gly	Gly	Ser	Leu	Ala	Ser	Gly	Gly	Pro	Gly	Pro	Gly		
835					840					845						
His	Ala	Ser	Leu	Ser	Gln	Arg	Leu	Arg	Leu	Tyr	Leu	Ala	Asp	Ser	Trp	
850					855					860						
Asn	Gln	Cys	Asp	Leu	Val	Ala	Leu	Thr	Cys	Phe	Leu	Leu	Gly	Val	Gly	
865					870					875					880	
Cys	Arg	Leu	Thr	Pro	Gly	Leu	Tyr	His	Leu	Gly	Arg	Thr	Val	Leu	Cys	
885					890					895						
Ile	Asp	Phe	Met	Val	Phe	Thr	Val	Arg	Leu	Leu	His	Ile	Phe	Thr	Val	
900					905					910						
Asn	Lys	Gln	Leu	Gly	Pro	Lys	Ile	Val	Ile	Val	Ser	Lys	Met	Met	Lys	
915					920					925						
Asp	Val	Phe	Phe	Phe	Leu	Phe	Leu	Gly	Val	Trp	Leu	Val	Ala	Tyr		
930					935					940						
Gly	Val	Ala	Thr	Glu	Gly	Leu	Leu	Arg	Pro	Arg	Asp	Ser	Asp	Phe	Pro	
945					950					955					960	
Ser	Ile	Leu	Arg	Arg	Val	Phe	Tyr	Arg	Pro	Tyr	Leu	Gln	Ile	Phe	Gly	
965					970					975						
Gln	Ile	Pro	Gln	Glu	Asp	Met	Asp	Val	Ala	Leu	Met	Glu	His	Ser	Asn	
980					985					990						
Cys	Ser	Ser	Glu	Pro	Gly	Phe	Trp	Ala	His	Pro	Pro	Gly	Ala	Gln	Ala	
995					1000					1005						
Gly	Thr	Cys	Val	Ser	Gln	Tyr	Ala	Asn	Trp	Leu	Val	Val	Leu	Leu	Leu	
1010					1015					1020						
Val	Ile	Phe	Leu	Leu	Val	Ala	Asn	Ile	Leu	Leu	Val	Asn	Leu	Leu	Ile	
1025					1030					1035					1040	
Ala	Met	Phe	Ser	Tyr	Thr	Phe	Gly	Lys	Val	Gln	Gly	Asn	Ser	Asp	Leu	
1045					1050					1055						
Tyr	Trp	Lys	Ala	Gln	Arg	Tyr	Arg	Leu	Ile	Arg	Glu	Phe	His	Ser	Arg	
1060					1065					1070						
Pro	Ala	Leu	Ala	Pro	Pro	Phe	Ile	Val	Ile	Ser	His	Leu	Arg	Leu	Leu	
1075					1080					1085						
Leu	Arg	Gln	Leu	Cys	Arg	Arg	Pro	Arg	Ser	Pro	Gln	Pro	Ser	Ser	Pro	
1090					1095					1100						
Ala	Leu	Glu	His	Phe	Arg	Val	Tyr	Leu	Ser	Lys	Glu	Ala	Glu	Arg	Lys	
1105					1110					1115					1120	
Leu	Leu	Thr	Trp	Glu	Ser	Val	His	Lys	Glu	Asn	Phe	Leu	Leu	Ala	Arg	
1125					1130					1135						
Ala	Arg	Asp	Lys	Arg	Glu	Ser	Asp	Ser	Glu	Arg	Leu	Lys	Arg	Thr	Ser	
1140					1145					1150						
Gln	Lys	Val	Asp	Leu	Ala	Leu	Lys	Gln	Leu	Gly	His	Ile	Arg	Glu	Tyr	
1155					1160					1165						

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Glu Gln Arg Leu Lys Val Leu Glu Arg Glu Val Gln Gln Cys Ser Arg
1170 1175 1180

Val Leu Gly Trp Val Ala Glu Ala Leu Ser Arg Ser Ala Leu Leu Pro
1185 1190 1195 1200

Pro Gly Gly Pro Pro Pro Pro Asp Leu Pro Gly Ser Lys Asp
1205 1210

<210> SEQ ID NO 13
<211> LENGTH: 188
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: TDGF1

<400> SEQUENCE: 13

Met Asp Cys Arg Lys Met Ala Arg Phe Ser Tyr Ser Val Ile Trp Ile
1 5 10 15

Met Ala Ile Ser Lys Val Phe Glu Leu Gly Leu Val Ala Gly Leu Gly
20 25 30

His Gln Glu Phe Ala Arg Pro Ser Arg Gly Tyr Leu Ala Phe Arg Asp
35 40 45

Asp Ser Ile Trp Pro Gln Glu Glu Pro Ala Ile Arg Pro Arg Ser Ser
50 55 60

Gln Arg Val Pro Pro Met Gly Ile Gln His Ser Lys Glu Leu Asn Arg
65 70 75 80

Thr Cys Cys Leu Asn Gly Gly Thr Cys Met Leu Gly Ser Phe Cys Ala
85 90 95

Cys Pro Pro Ser Phe Tyr Gly Arg Asn Cys Glu His Asp Val Arg Lys
100 105 110

Glu Asn Cys Gly Ser Val Pro His Asp Thr Trp Leu Pro Lys Lys Cys
115 120 125

Ser Leu Cys Lys Cys Trp His Gly Gln Leu Arg Cys Phe Pro Gln Ala
130 135 140

Phe Leu Pro Gly Cys Asp Gly Leu Val Met Asp Glu His Leu Val Ala
145 150 155 160

Ser Arg Thr Pro Glu Leu Pro Pro Ser Ala Arg Thr Thr Thr Phe Met
165 170 175

Leu Val Gly Ile Cys Leu Ser Ile Gln Ser Tyr Tyr
180 185

<210> SEQ ID NO 14
<211> LENGTH: 1033
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: CD21

<400> SEQUENCE: 14

Met Gly Ala Ala Gly Leu Leu Gly Val Phe Leu Ala Leu Val Ala Pro
1 5 10 15

Gly Val Leu Gly Ile Ser Cys Gly Ser Pro Pro Pro Ile Leu Asn Gly
20 25 30

Arg Ile Ser Tyr Tyr Ser Thr Pro Ile Ala Val Gly Thr Val Ile Arg
35 40 45

Tyr Ser Cys Ser Gly Thr Phe Arg Leu Ile Gly Glu Lys Ser Leu Leu
50 55 60

Cys Ile Thr Lys Asp Lys Val Asp Gly Thr Trp Asp Lys Pro Ala Pro

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65	70	75	80
Lys Cys Glu Tyr Phe Asn Lys Tyr Ser Ser Cys Pro Glu Pro Ile Val	85	90	95
Pro Gly Gly Tyr Lys Ile Arg Gly Ser Thr Pro Tyr Arg His Gly Asp	100	105	110
Ser Val Thr Phe Ala Cys Lys Thr Asn Phe Ser Met Asn Gly Asn Lys	115	120	125
Ser Val Trp Cys Gln Ala Asn Asn Met Trp Gly Pro Thr Arg Leu Pro	130	135	140
Thr Cys Val Ser Val Phe Pro Leu Glu Cys Pro Ala Leu Pro Met Ile	145	150	155
His Asn Gly His His Thr Ser Glu Asn Val Gly Ser Ile Ala Pro Gly	165	170	175
Leu Ser Val Thr Tyr Ser Cys Glu Ser Gly Tyr Leu Leu Val Gly Glu	180	185	190
Lys Ile Ile Asn Cys Leu Ser Ser Gly Lys Trp Ser Ala Val Pro Pro	195	200	205
Thr Cys Glu Glu Ala Arg Cys Lys Ser Leu Gly Arg Phe Pro Asn Gly	210	215	220
Lys Val Lys Glu Pro Pro Ile Leu Arg Val Gly Val Thr Ala Asn Phe	225	230	235
Phe Cys Asp Glu Gly Tyr Arg Leu Gln Gly Pro Pro Ser Ser Arg Cys	245	250	255
Val Ile Ala Gly Gln Gly Val Ala Trp Thr Lys Met Pro Val Cys Glu	260	265	270
Glu Ile Phe Cys Pro Ser Pro Pro Pro Ile Leu Asn Gly Arg His Ile	275	280	285
Gly Asn Ser Leu Ala Asn Val Ser Tyr Gly Ser Ile Val Thr Tyr Thr	290	295	300
Cys Asp Pro Asp Pro Glu Glu Gly Val Asn Phe Ile Leu Ile Gly Glu	305	310	315
Ser Thr Leu Arg Cys Thr Val Asp Ser Gln Lys Thr Gly Thr Trp Ser	325	330	335
Gly Pro Ala Pro Arg Cys Glu Leu Ser Thr Ser Ala Val Gln Cys Pro	340	345	350
His Pro Gln Ile Leu Arg Gly Arg Met Val Ser Gly Gln Lys Asp Arg	355	360	365
Tyr Thr Tyr Asn Asp Thr Val Ile Phe Ala Cys Met Phe Gly Phe Thr	370	375	380
Leu Lys Gly Ser Lys Gln Ile Arg Cys Asn Ala Gln Gly Thr Trp Glu	385	390	395
Pro Ser Ala Pro Val Cys Glu Lys Glu Cys Gln Ala Pro Pro Asn Ile	405	410	415
Leu Asn Gly Gln Lys Glu Asp Arg His Met Val Arg Phe Asp Pro Gly	420	425	430
Thr Ser Ile Lys Tyr Ser Cys Asn Pro Gly Tyr Val Leu Val Gly Glu	435	440	445
Glu Ser Ile Gln Cys Thr Ser Glu Gly Val Trp Thr Pro Pro Val Pro	450	455	460
Gln Cys Lys Val Ala Ala Cys Glu Ala Thr Gly Arg Gln Leu Leu Thr	465	470	475
Lys Pro Gln His Gln Phe Val Arg Pro Asp Val Asn Ser Ser Cys Gly	485	490	495

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Glu Gly Tyr Lys Leu Ser Gly Ser Val Tyr Gln Glu Cys Gln Gly Thr
 500 505 510
 Ile Pro Trp Phe Met Glu Ile Arg Leu Cys Lys Glu Ile Thr Cys Pro
 515 520 525
 Pro Pro Pro Val Ile Tyr Asn Gly Ala His Thr Gly Ser Ser Leu Glu
 530 535 540
 Asp Phe Pro Tyr Gly Thr Thr Val Thr Tyr Thr Cys Asn Pro Gly Pro
 545 550 555 560
 Glu Arg Gly Val Glu Phe Ser Leu Ile Gly Glu Ser Thr Ile Arg Cys
 565 570 575
 Thr Ser Asn Asp Gln Glu Arg Gly Thr Trp Ser Gly Pro Ala Pro Leu
 580 585 590
 Cys Lys Leu Ser Leu Leu Ala Val Gln Cys Ser His Val His Ile Ala
 595 600 605
 Asn Gly Tyr Lys Ile Ser Gly Lys Glu Ala Pro Tyr Phe Tyr Asn Asp
 610 615 620
 Thr Val Thr Phe Lys Cys Tyr Ser Gly Phe Thr Leu Lys Gly Ser Ser
 625 630 635 640
 Gln Ile Arg Cys Lys Ala Asp Asn Thr Trp Asp Pro Glu Ile Pro Val
 645 650 655
 Cys Glu Lys Glu Thr Cys Gln His Val Arg Gln Ser Leu Gln Glu Leu
 660 665 670
 Pro Ala Gly Ser Arg Val Glu Leu Val Asn Thr Ser Cys Gln Asp Gly
 675 680 685
 Tyr Gln Leu Thr Gly His Ala Tyr Gln Met Cys Gln Asp Ala Glu Asn
 690 695 700
 Gly Ile Trp Phe Lys Lys Ile Pro Leu Cys Lys Val Ile His Cys His
 705 710 715 720
 Pro Pro Pro Val Ile Val Asn Gly Lys His Thr Gly Met Met Ala Glu
 725 730 735
 Asn Phe Leu Tyr Gly Asn Glu Val Ser Tyr Glu Cys Asp Gln Gly Phe
 740 745 750
 Tyr Leu Leu Gly Glu Lys Lys Leu Gln Cys Arg Ser Asp Ser Lys Gly
 755 760 765
 His Gly Ser Trp Ser Gly Pro Ser Pro Gln Cys Leu Arg Ser Pro Pro
 770 775 780
 Val Thr Arg Cys Pro Asn Pro Glu Val Lys His Gly Tyr Lys Leu Asn
 785 790 795 800
 Lys Thr His Ser Ala Tyr Ser His Asn Asp Ile Val Tyr Val Asp Cys
 805 810 815
 Asn Pro Gly Phe Ile Met Asn Gly Ser Arg Val Ile Arg Cys His Thr
 820 825 830
 Asp Asn Thr Trp Val Pro Gly Val Pro Thr Cys Ile Lys Lys Ala Phe
 835 840 845
 Ile Gly Cys Pro Pro Pro Lys Thr Pro Asn Gly Asn His Thr Gly
 850 855 860
 Gly Asn Ile Ala Arg Phe Ser Pro Gly Met Ser Ile Leu Tyr Ser Cys
 865 870 875 880
 Asp Gln Gly Tyr Leu Leu Val Gly Glu Ala Leu Leu Leu Cys Thr His
 885 890 895
 Glu Gly Thr Trp Ser Gln Pro Ala Pro His Cys Lys Glu Val Asn Cys
 900 905 910

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Ser Ser Pro Ala Asp Met Asp Gly Ile Gln Lys Gly Leu Glu Pro Arg
915 920 925

Lys Met Tyr Gln Tyr Gly Ala Val Val Thr Leu Glu Cys Glu Asp Gly
930 935 940

Tyr Met Leu Glu Gly Ser Pro Gln Ser Gln Cys Gln Ser Asp His Gln
945 950 955 960

Trp Asn Pro Pro Leu Ala Val Cys Arg Ser Arg Ser Leu Ala Pro Val
965 970 975

Leu Cys Gly Ile Ala Ala Gly Leu Ile Leu Thr Phe Leu Ile Val
980 985 990

Ile Thr Leu Tyr Val Ile Ser Lys His Arg Glu Arg Asn Tyr Tyr Thr
995 1000 1005

Asp Thr Ser Gln Lys Glu Ala Phe His Leu Glu Ala Arg Glu Val Tyr
1010 1015 1020

Ser Val Asp Pro Tyr Asn Pro Ala Ser
1025 1030

<210> SEQ ID NO 15
<211> LENGTH: 229
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: CD79B

<400> SEQUENCE: 15

Met Ala Arg Leu Ala Leu Ser Pro Val Pro Ser His Trp Met Val Ala
1 5 10 15

Leu Leu Leu Leu Leu Ser Ala Glu Pro Val Pro Ala Ala Arg Ser Glu
20 25 30

Asp Arg Tyr Arg Asn Pro Lys Gly Ser Ala Cys Ser Arg Ile Trp Gln
35 40 45

Ser Pro Arg Phe Ile Ala Arg Lys Arg Gly Phe Thr Val Lys Met His
50 55 60

Cys Tyr Met Asn Ser Ala Ser Gly Asn Val Ser Trp Leu Trp Lys Gln
65 70 75 80

Glu Met Asp Glu Asn Pro Gln Gln Leu Lys Leu Glu Lys Gly Arg Met
85 90 95

Glu Glu Ser Gln Asn Glu Ser Leu Ala Thr Leu Thr Ile Gln Gly Ile
100 105 110

Arg Phe Glu Asp Asn Gly Ile Tyr Phe Cys Gln Gln Lys Cys Asn Asn
115 120 125

Thr Ser Glu Val Tyr Gln Gly Cys Gly Thr Glu Leu Arg Val Met Gly
130 135 140

Phe Ser Thr Leu Ala Gln Leu Lys Gln Arg Asn Thr Leu Lys Asp Gly
145 150 155 160

Ile Ile Met Ile Gln Thr Leu Leu Ile Ile Leu Phe Ile Ile Val Pro
165 170 175

Ile Phe Leu Leu Leu Asp Lys Asp Asp Ser Lys Ala Gly Met Glu Glu
180 185 190

Asp His Thr Tyr Glu Gly Leu Asp Ile Asp Gln Thr Ala Thr Tyr Glu
195 200 205

Asp Ile Val Thr Leu Arg Thr Gly Glu Val Lys Trp Ser Val Gly Glu
210 215 220

His Pro Gly Gln Glu
225

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<210> SEQ ID NO 16
<211> LENGTH: 508
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: FcRH2

<400> SEQUENCE: 16

Met Leu Leu Trp Ser Leu Leu Val Ile Phe Asp Ala Val Thr Glu Gln
 1             5             10             15

Ala Asp Ser Leu Thr Leu Val Ala Pro Ser Ser Val Phe Glu Gly Asp
 20             25             30

Ser Ile Val Leu Lys Cys Gln Gly Glu Gln Asn Trp Lys Ile Gln Lys
 35             40             45

Met Ala Tyr His Lys Asp Asn Lys Glu Leu Ser Val Phe Lys Lys Phe
 50             55             60

Ser Asp Phe Leu Ile Gln Ser Ala Val Leu Ser Asp Ser Gly Asn Tyr
 65             70             75             80

Phe Cys Ser Thr Lys Gly Gln Leu Phe Leu Trp Asp Lys Thr Ser Asn
 85             90             95

Ile Val Lys Ile Lys Val Gln Glu Leu Phe Gln Arg Pro Val Leu Thr
100            105            110

Ala Ser Ser Phe Gln Pro Ile Glu Gly Gly Pro Val Ser Leu Lys Cys
115            120            125

Glu Thr Arg Leu Ser Pro Gln Arg Leu Asp Val Gln Leu Gln Phe Cys
130            135            140

Phe Phe Arg Glu Asn Gln Val Leu Gly Ser Gly Trp Ser Ser Ser Pro
145            150            155            160

Glu Leu Gln Ile Ser Ala Val Trp Ser Glu Asp Thr Gly Ser Tyr Trp
165            170            175

Cys Lys Ala Glu Thr Val Thr His Arg Ile Arg Lys Gln Ser Leu Gln
180            185            190

Ser Gln Ile His Val Gln Arg Ile Pro Ile Ser Asn Val Ser Leu Glu
195            200            205

Ile Arg Ala Pro Gly Gly Gln Val Thr Glu Gly Gln Lys Leu Ile Leu
210            215            220

Leu Cys Ser Val Ala Gly Gly Thr Gly Asn Val Thr Phe Ser Trp Tyr
225            230            235            240

Arg Glu Ala Thr Gly Thr Ser Met Gly Lys Lys Thr Gln Arg Ser Leu
245            250            255

Ser Ala Glu Leu Glu Ile Pro Ala Val Lys Glu Ser Asp Ala Gly Lys
260            265            270

Tyr Tyr Cys Arg Ala Asp Asn Gly His Val Pro Ile Gln Ser Lys Val
275            280            285

Val Asn Ile Pro Val Arg Ile Pro Val Ser Arg Pro Val Leu Thr Leu
290            295            300

Arg Ser Pro Gly Ala Gln Ala Ala Val Gly Asp Leu Leu Glu Leu His
305            310            315            320

Cys Glu Ala Leu Arg Gly Ser Pro Pro Ile Leu Tyr Gln Phe Tyr His
325            330            335

Glu Asp Val Thr Leu Gly Asn Ser Ser Ala Pro Ser Gly Gly Gly Ala
340            345            350

Ser Phe Asn Leu Ser Leu Thr Ala Glu His Ser Gly Asn Tyr Ser Cys
355            360            365

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Glu Ala Asn Asn Gly Leu Gly Ala Gln Cys Ser Glu Ala Val Pro Val
 370 375 380
 Ser Ile Ser Gly Pro Asp Gly Tyr Arg Arg Asp Leu Met Thr Ala Gly
 385 390 395 400
 Val Leu Trp Gly Leu Phe Gly Val Leu Gly Phe Thr Gly Val Ala Leu
 405 410 415
 Leu Leu Tyr Ala Leu Phe His Lys Ile Ser Gly Glu Ser Ser Ala Thr
 420 425 430
 Asn Glu Pro Arg Gly Ala Ser Arg Pro Asn Pro Gln Glu Phe Thr Tyr
 435 440 445
 Ser Ser Pro Thr Pro Asp Met Glu Glu Leu Gln Pro Val Tyr Val Asn
 450 455 460
 Val Gly Ser Val Asp Val Asp Val Val Tyr Ser Gln Val Trp Ser Met
 465 470 475 480
 Gln Gln Pro Glu Ser Ser Ala Asn Ile Arg Thr Leu Leu Glu Asn Lys
 485 490 495
 Asp Ser Gln Val Ile Tyr Ser Ser Val Lys Lys Ser
 500 505

<210> SEQ ID NO 17
 <211> LENGTH: 1255
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <223> OTHER INFORMATION: HER2

<400> SEQUENCE: 17

Met Glu Leu Ala Ala Leu Cys Arg Trp Gly Leu Leu Leu Ala Leu Leu
 1 5 10 15
 Pro Pro Gly Ala Ala Ser Thr Gln Val Cys Thr Gly Thr Asp Met Lys
 20 25 30
 Leu Arg Leu Pro Ala Ser Pro Glu Thr His Leu Asp Met Leu Arg His
 35 40 45
 Leu Tyr Gln Gly Cys Gln Val Val Gln Gly Asn Leu Glu Leu Thr Tyr
 50 55 60
 Leu Pro Thr Asn Ala Ser Leu Ser Phe Leu Gln Asp Ile Gln Glu Val
 65 70 75 80
 Gln Gly Tyr Val Leu Ile Ala His Asn Gln Val Arg Gln Val Pro Leu
 85 90 95
 Gln Arg Leu Arg Ile Val Arg Gly Thr Gln Leu Phe Glu Asp Asn Tyr
 100 105 110
 Ala Leu Ala Val Leu Asp Asn Gly Asp Pro Leu Asn Asn Thr Thr Pro
 115 120 125
 Val Thr Gly Ala Ser Pro Gly Gly Leu Arg Glu Leu Gln Leu Arg Ser
 130 135 140
 Leu Thr Glu Ile Leu Lys Gly Gly Val Leu Ile Gln Arg Asn Pro Gln
 145 150 155 160
 Leu Cys Tyr Gln Asp Thr Ile Leu Trp Lys Asp Ile Phe His Lys Asn
 165 170 175
 Asn Gln Leu Ala Leu Thr Leu Ile Asp Thr Asn Arg Ser Arg Ala Cys
 180 185 190
 His Pro Cys Ser Pro Met Cys Lys Gly Ser Arg Cys Trp Gly Glu Ser
 195 200 205
 Ser Glu Asp Cys Gln Ser Leu Thr Arg Thr Val Cys Ala Gly Gly Cys
 210 215 220

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Ala Arg Cys Lys Gly Pro Leu Pro Thr Asp Cys Cys His Glu Gln Cys
 225 230 235 240
 Ala Ala Gly Cys Thr Gly Pro Lys His Ser Asp Cys Leu Ala Cys Leu
 245 250 255
 His Phe Asn His Ser Gly Ile Cys Glu Leu His Cys Pro Ala Leu Val
 260 265 270
 Thr Tyr Asn Thr Asp Thr Phe Glu Ser Met Pro Asn Pro Glu Gly Arg
 275 280 285
 Tyr Thr Phe Gly Ala Ser Cys Val Thr Ala Cys Pro Tyr Asn Tyr Leu
 290 295 300
 Ser Thr Asp Val Gly Ser Cys Thr Leu Val Cys Pro Leu His Asn Gln
 305 310 315 320
 Glu Val Thr Ala Glu Asp Gly Thr Gln Arg Cys Glu Lys Cys Ser Lys
 325 330 335
 Pro Cys Ala Arg Val Cys Tyr Gly Leu Gly Met Glu His Leu Arg Glu
 340 345 350
 Val Arg Ala Val Thr Ser Ala Asn Ile Gln Glu Phe Ala Gly Cys Lys
 355 360 365
 Lys Ile Phe Gly Ser Leu Ala Phe Leu Pro Glu Ser Phe Asp Gly Asp
 370 375 380
 Pro Ala Ser Asn Thr Ala Pro Leu Gln Pro Glu Gln Leu Gln Val Phe
 385 390 395 400
 Glu Thr Leu Glu Glu Ile Thr Gly Tyr Leu Tyr Ile Ser Ala Trp Pro
 405 410 415
 Asp Ser Leu Pro Asp Leu Ser Val Phe Gln Asn Leu Gln Val Ile Arg
 420 425 430
 Gly Arg Ile Leu His Asn Gly Ala Tyr Ser Leu Thr Leu Gln Gly Leu
 435 440 445
 Gly Ile Ser Trp Leu Gly Leu Arg Ser Leu Arg Glu Leu Gly Ser Gly
 450 455 460
 Leu Ala Leu Ile His His Asn Thr His Leu Cys Phe Val His Thr Val
 465 470 475 480
 Pro Trp Asp Gln Leu Phe Arg Asn Pro His Gln Ala Leu Leu His Thr
 485 490 495
 Ala Asn Arg Pro Glu Asp Glu Cys Val Gly Glu Gly Leu Ala Cys His
 500 505 510
 Gln Leu Cys Ala Arg Gly His Cys Trp Gly Pro Gly Pro Thr Gln Cys
 515 520 525
 Val Asn Cys Ser Gln Phe Leu Arg Gly Gln Glu Cys Val Glu Glu Cys
 530 535 540
 Arg Val Leu Gln Gly Leu Pro Arg Glu Tyr Val Asn Ala Arg His Cys
 545 550 555 560
 Leu Pro Cys His Pro Glu Cys Gln Pro Gln Asn Gly Ser Val Thr Cys
 565 570 575
 Phe Gly Pro Glu Ala Asp Gln Cys Val Ala Cys Ala His Tyr Lys Asp
 580 585 590
 Pro Pro Phe Cys Val Ala Arg Cys Pro Ser Gly Val Lys Pro Asp Leu
 595 600 605
 Ser Tyr Met Pro Ile Trp Lys Phe Pro Asp Glu Glu Gly Ala Cys Gln
 610 615 620
 Pro Cys Pro Ile Asn Cys Thr His Ser Cys Val Asp Leu Asp Asp Lys
 625 630 635 640
 Gly Cys Pro Ala Glu Gln Arg Ala Ser Pro Leu Thr Ser Ile Ile Ser

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645					650					655					
Ala	Val	Val	Gly	Ile	Leu	Leu	Val	Val	Val	Leu	Gly	Val	Val	Phe	Gly
			660					665					670		
Ile	Leu	Ile	Lys	Arg	Arg	Gln	Gln	Lys	Ile	Arg	Lys	Tyr	Thr	Met	Arg
		675					680					685			
Arg	Leu	Leu	Gln	Glu	Thr	Glu	Leu	Val	Glu	Pro	Leu	Thr	Pro	Ser	Gly
		690					695					700			
Ala	Met	Pro	Asn	Gln	Ala	Gln	Met	Arg	Ile	Leu	Lys	Glu	Thr	Glu	Leu
	705			710							715				720
Arg	Lys	Val	Lys	Val	Leu	Gly	Ser	Gly	Ala	Phe	Gly	Thr	Val	Tyr	Lys
			725						730					735	
Gly	Ile	Trp	Ile	Pro	Asp	Gly	Glu	Asn	Val	Lys	Ile	Pro	Val	Ala	Ile
			740					745					750		
Lys	Val	Leu	Arg	Glu	Asn	Thr	Ser	Pro	Lys	Ala	Asn	Lys	Glu	Ile	Leu
		755					760					765			
Asp	Glu	Ala	Tyr	Val	Met	Ala	Gly	Val	Gly	Ser	Pro	Tyr	Val	Ser	Arg
	770					775					780				
Leu	Leu	Gly	Ile	Cys	Leu	Thr	Ser	Thr	Val	Gln	Leu	Val	Thr	Gln	Leu
	785			790							795				800
Met	Pro	Tyr	Gly	Cys	Leu	Leu	Asp	His	Val	Arg	Glu	Asn	Arg	Gly	Arg
			805					810						815	
Leu	Gly	Ser	Gln	Asp	Leu	Leu	Asn	Trp	Cys	Met	Gln	Ile	Ala	Lys	Gly
			820					825					830		
Met	Ser	Tyr	Leu	Glu	Asp	Val	Arg	Leu	Val	His	Arg	Asp	Leu	Ala	Ala
		835					840					845			
Arg	Asn	Val	Leu	Val	Lys	Ser	Pro	Asn	His	Val	Lys	Ile	Thr	Asp	Phe
	850						855					860			
Gly	Leu	Ala	Arg	Leu	Leu	Asp	Ile	Asp	Glu	Thr	Glu	Tyr	His	Ala	Asp
	865			870					875						880
Gly	Gly	Lys	Val	Pro	Ile	Lys	Trp	Met	Ala	Leu	Glu	Ser	Ile	Leu	Arg
			885						890					895	
Arg	Arg	Phe	Thr	His	Gln	Ser	Asp	Val	Trp	Ser	Tyr	Gly	Val	Thr	Val
			900					905					910		
Trp	Glu	Leu	Met	Thr	Phe	Gly	Ala	Lys	Pro	Tyr	Asp	Gly	Ile	Pro	Ala
		915					920					925			
Arg	Glu	Ile	Pro	Asp	Leu	Leu	Glu	Lys	Gly	Glu	Arg	Leu	Pro	Gln	Pro
		930					935					940			
Pro	Ile	Cys	Thr	Ile	Asp	Val	Tyr	Met	Ile	Met	Val	Lys	Cys	Trp	Met
	945			950							955				960
Ile	Asp	Ser	Glu	Cys	Arg	Pro	Arg	Phe	Arg	Glu	Leu	Val	Ser	Glu	Phe
			965					970						975	
Ser	Arg	Met	Ala	Arg	Asp	Pro	Gln	Arg	Phe	Val	Val	Ile	Gln	Asn	Glu
			980					985						990	
Asp	Leu	Gly	Pro	Ala	Ser	Pro	Leu	Asp	Ser	Thr	Phe	Tyr	Arg	Ser	Leu
		995					1000					1005			
Leu	Glu	Asp	Asp	Asp	Met	Gly	Asp	Leu	Val	Asp	Ala	Glu	Glu	Tyr	Leu
	1010					1015					1020				
Val	Pro	Gln	Gln	Gly	Phe	Phe	Cys	Pro	Asp	Pro	Ala	Pro	Gly	Ala	Gly
	1025					1030					1035				1040
Gly	Met	Val	His	His	Arg	His	Arg	Ser	Ser	Ser	Thr	Arg	Ser	Gly	Gly
			1045					1050						1055	
Gly	Asp	Leu	Thr	Leu	Gly	Leu	Glu	Pro	Ser	Glu	Glu	Glu	Ala	Pro	Arg
			1060					1065					1070		

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Ser Pro Leu Ala Pro Ser Glu Gly Ala Gly Ser Asp Val Phe Asp Gly
1075 1080 1085

Asp Leu Gly Met Gly Ala Ala Lys Gly Leu Gln Ser Leu Pro Thr His
1090 1095 1100

Asp Pro Ser Pro Leu Gln Arg Tyr Ser Glu Asp Pro Thr Val Pro Leu
1105 1110 1115 1120

Pro Ser Glu Thr Asp Gly Tyr Val Ala Pro Leu Thr Cys Ser Pro Gln
1125 1130 1135

Pro Glu Tyr Val Asn Gln Pro Asp Val Arg Pro Gln Pro Pro Ser Pro
1140 1145 1150

Arg Glu Gly Pro Leu Pro Ala Ala Arg Pro Ala Gly Ala Thr Leu Glu
1155 1160 1165

Arg Pro Lys Thr Leu Ser Pro Gly Lys Asn Gly Val Val Lys Asp Val
1170 1175 1180

Phe Ala Phe Gly Gly Ala Val Glu Asn Pro Glu Tyr Leu Thr Pro Gln
1185 1190 1195 1200

Gly Gly Ala Ala Pro Gln Pro His Pro Pro Pro Ala Phe Ser Pro Ala
1205 1210 1215

Phe Asp Asn Leu Tyr Tyr Trp Asp Gln Asp Pro Pro Glu Arg Gly Ala
1220 1225 1230

Pro Pro Ser Thr Phe Lys Gly Thr Pro Thr Ala Glu Asn Pro Glu Tyr
1235 1240 1245

Leu Gly Leu Asp Val Pro Val
1250 1255

<210> SEQ ID NO 18
 <211> LENGTH: 344
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <223> OTHER INFORMATION: CEACAM6

<400> SEQUENCE: 18

Met Gly Pro Pro Ser Ala Pro Pro Cys Arg Leu His Val Pro Trp Lys
1 5 10 15

Glu Val Leu Leu Thr Ala Ser Leu Leu Thr Phe Trp Asn Pro Pro Thr
20 25 30

Thr Ala Lys Leu Thr Ile Glu Ser Thr Pro Phe Asn Val Ala Glu Gly
35 40 45

Lys Glu Val Leu Leu Ala His Asn Leu Pro Gln Asn Arg Ile Gly
50 55 60

Tyr Ser Trp Tyr Lys Gly Glu Arg Val Asp Gly Asn Ser Leu Ile Val
65 70 75 80

Gly Tyr Val Ile Gly Thr Gln Gln Ala Thr Pro Gly Pro Ala Tyr Ser
85 90 95

Gly Arg Glu Thr Ile Tyr Pro Asn Ala Ser Leu Leu Ile Gln Asn Val
100 105 110

Thr Gln Asn Asp Thr Gly Phe Tyr Thr Leu Gln Val Ile Lys Ser Asp
115 120 125

Leu Val Asn Glu Glu Ala Thr Gly Gln Phe His Val Tyr Pro Glu Leu
130 135 140

Pro Lys Pro Ser Ile Ser Ser Asn Asn Ser Asn Pro Val Glu Asp Lys
145 150 155 160

Asp Ala Val Ala Phe Thr Cys Glu Pro Glu Val Gln Asn Thr Thr Tyr
165 170 175

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Leu Trp Trp Val Asn Gly Gln Ser Leu Pro Val Ser Pro Arg Leu Gln
 180 185 190
 Leu Ser Asn Gly Asn Met Thr Leu Thr Leu Leu Ser Val Lys Arg Asn
 195 200 205
 Asp Ala Gly Ser Tyr Glu Cys Glu Ile Gln Asn Pro Ala Ser Ala Asn
 210 215 220
 Arg Ser Asp Pro Val Thr Leu Asn Val Leu Tyr Gly Pro Asp Val Pro
 225 230 235 240
 Thr Ile Ser Pro Ser Lys Ala Asn Tyr Arg Pro Gly Glu Asn Leu Asn
 245 250 255
 Leu Ser Cys His Ala Ala Ser Asn Pro Pro Ala Gln Tyr Ser Trp Phe
 260 265 270
 Ile Asn Gly Thr Phe Gln Gln Ser Thr Gln Glu Leu Phe Ile Pro Asn
 275 280 285
 Ile Thr Val Asn Asn Ser Gly Ser Tyr Met Cys Gln Ala His Asn Ser
 290 295 300
 Ala Thr Gly Leu Asn Arg Thr Thr Val Thr Met Ile Thr Val Ser Gly
 305 310 315 320
 Ser Ala Pro Val Leu Ser Ala Val Ala Thr Val Gly Ile Thr Ile Gly
 325 330 335
 Val Leu Ala Arg Val Ala Leu Ile
 340

<210> SEQ ID NO 19
 <211> LENGTH: 411
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <223> OTHER INFORMATION: DPEP1

<400> SEQUENCE: 19

Met Trp Ser Gly Trp Trp Leu Trp Pro Leu Val Ala Val Cys Thr Ala
 1 5 10 15
 Asp Phe Phe Arg Asp Glu Ala Glu Arg Ile Met Arg Asp Ser Pro Val
 20 25 30
 Ile Asp Gly His Asn Asp Leu Pro Trp Gln Leu Leu Asp Met Phe Asn
 35 40 45
 Asn Arg Leu Gln Asp Glu Arg Ala Asn Leu Thr Thr Leu Ala Gly Thr
 50 55 60
 His Thr Asn Ile Pro Lys Leu Arg Ala Gly Phe Val Gly Gly Gln Phe
 65 70 75 80
 Trp Ser Val Tyr Thr Pro Cys Asp Thr Gln Asn Lys Asp Ala Val Arg
 85 90 95
 Arg Thr Leu Glu Gln Met Asp Val Val His Arg Met Cys Arg Met Tyr
 100 105 110
 Pro Glu Thr Phe Leu Tyr Val Thr Ser Ser Ala Gly Ile Arg Gln Ala
 115 120 125
 Phe Arg Glu Gly Lys Val Ala Ser Leu Ile Gly Val Glu Gly Gly His
 130 135 140
 Ser Ile Asp Ser Ser Leu Gly Val Leu Arg Ala Leu Tyr Gln Leu Gly
 145 150 155 160
 Met Arg Tyr Leu Thr Leu Thr His Ser Cys Asn Thr Pro Trp Ala Asp
 165 170 175
 Asn Trp Leu Val Asp Thr Gly Asp Ser Glu Pro Gln Ser Gln Gly Leu
 180 185 190

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Ser Pro Phe Gly Gln Arg Val Val Lys Glu Leu Asn Arg Leu Gly Val
195 200 205

Leu Ile Asp Leu Ala His Val Ser Val Ala Thr Met Lys Ala Thr Leu
210 215 220

Gln Leu Ser Arg Ala Pro Val Ile Phe Ser His Ser Ser Ala Tyr Ser
225 230 235 240

Val Cys Ala Ser Arg Arg Asn Val Pro Asp Asp Val Leu Arg Leu Val
245 250 255

Lys Gln Thr Asp Ser Leu Val Met Val Asn Phe Tyr Asn Asn Tyr Ile
260 265 270

Ser Cys Thr Asn Lys Ala Asn Leu Ser Gln Val Ala Asp His Leu Asp
275 280 285

His Ile Lys Glu Val Ala Gly Ala Arg Ala Val Gly Phe Gly Gly Asp
290 295 300

Phe Asp Gly Val Pro Arg Val Pro Glu Gly Leu Glu Asp Val Ser Lys
305 310 315 320

Tyr Pro Asp Leu Ile Ala Glu Leu Leu Arg Arg Asn Trp Thr Glu Ala
325 330 335

Glu Val Lys Gly Ala Leu Ala Asp Asn Leu Leu Arg Val Phe Glu Ala
340 345 350

Val Glu Gln Ala Ser Asn Leu Thr Gln Ala Pro Glu Glu Glu Pro Ile
355 360 365

Pro Leu Asp Gln Leu Gly Gly Ser Cys Arg Thr His Tyr Gly Tyr Ser
370 375 380

Ser Gly Ala Ser Ser Leu His Arg His Trp Gly Leu Leu Leu Ala Ser
385 390 395 400

Leu Ala Pro Leu Val Leu Cys Leu Ser Leu Leu
405 410

<210> SEQ ID NO 20
 <211> LENGTH: 553
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <223> OTHER INFORMATION: IL20Ra

<400> SEQUENCE: 20

Met Arg Ala Pro Gly Arg Pro Ala Leu Arg Pro Leu Pro Leu Pro Pro
1 5 10 15

Leu Leu Leu Leu Leu Ala Ala Pro Trp Gly Arg Ala Val Pro Cys
20 25 30

Val Ser Gly Gly Leu Pro Lys Pro Ala Asn Ile Thr Phe Leu Ser Ile
35 40 45

Asn Met Lys Asn Val Leu Gln Trp Thr Pro Pro Glu Gly Leu Gln Gly
50 55 60

Val Lys Val Thr Tyr Thr Val Gln Tyr Phe Ile Tyr Gly Gln Lys Lys
65 70 75 80

Trp Leu Asn Lys Ser Glu Cys Arg Asn Ile Asn Arg Thr Tyr Cys Asp
85 90 95

Leu Ser Ala Glu Thr Ser Asp Tyr Glu His Gln Tyr Tyr Ala Lys Val
100 105 110

Lys Ala Ile Trp Gly Thr Lys Cys Ser Lys Trp Ala Glu Ser Gly Arg
115 120 125

Phe Tyr Pro Phe Leu Glu Thr Gln Ile Gly Pro Pro Glu Val Ala Leu
130 135 140

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Thr Thr Asp Glu Lys Ser Ile Ser Val Val Leu Thr Ala Pro Glu Lys
 145 150 155 160
 Trp Lys Arg Asn Pro Glu Asp Leu Pro Val Ser Met Gln Gln Ile Tyr
 165 170 175
 Ser Asn Leu Lys Tyr Asn Val Ser Val Leu Asn Thr Lys Ser Asn Arg
 180 185 190
 Thr Trp Ser Gln Cys Val Thr Asn His Thr Leu Val Leu Thr Trp Leu
 195 200 205
 Glu Pro Asn Thr Leu Tyr Cys Val His Val Glu Ser Phe Val Pro Gly
 210 215 220
 Pro Pro Arg Arg Ala Gln Pro Ser Glu Lys Gln Cys Ala Arg Thr Leu
 225 230 235 240
 Lys Asp Gln Ser Ser Glu Phe Lys Ala Lys Ile Ile Phe Trp Tyr Val
 245 250 255
 Leu Pro Ile Ser Ile Thr Val Phe Leu Phe Ser Val Met Gly Tyr Ser
 260 265 270
 Ile Tyr Arg Tyr Ile His Val Gly Lys Glu Lys His Pro Ala Asn Leu
 275 280 285
 Ile Leu Ile Tyr Gly Asn Glu Phe Asp Lys Arg Phe Phe Val Pro Ala
 290 295 300
 Glu Lys Ile Val Ile Asn Phe Ile Thr Leu Asn Ile Ser Asp Asp Ser
 305 310 315 320
 Lys Ile Ser His Gln Asp Met Ser Leu Leu Gly Lys Ser Ser Asp Val
 325 330 335
 Ser Ser Leu Asn Asp Pro Gln Pro Ser Gly Asn Leu Arg Pro Pro Gln
 340 345 350
 Glu Glu Glu Glu Val Lys His Leu Gly Tyr Ala Ser His Leu Met Glu
 355 360 365
 Ile Phe Cys Asp Ser Glu Glu Asn Thr Glu Gly Thr Ser Phe Thr Gln
 370 375 380
 Gln Glu Ser Leu Ser Arg Thr Ile Pro Pro Asp Lys Thr Val Ile Glu
 385 390 395 400
 Tyr Glu Tyr Asp Val Arg Thr Thr Asp Ile Cys Ala Gly Pro Glu Glu
 405 410 415
 Gln Glu Leu Ser Leu Gln Glu Glu Val Ser Thr Gln Gly Thr Leu Leu
 420 425 430
 Glu Ser Gln Ala Ala Leu Ala Val Leu Gly Pro Gln Thr Leu Gln Tyr
 435 440 445
 Ser Tyr Thr Pro Gln Leu Gln Asp Leu Asp Pro Leu Ala Gln Glu His
 450 455 460
 Thr Asp Ser Glu Glu Gly Pro Glu Glu Glu Pro Ser Thr Thr Leu Val
 465 470 475 480
 Asp Trp Asp Pro Gln Thr Gly Arg Leu Cys Ile Pro Ser Leu Ser Ser
 485 490 495
 Phe Asp Gln Asp Ser Glu Gly Cys Glu Pro Ser Glu Gly Asp Gly Leu
 500 505 510
 Gly Glu Glu Gly Leu Leu Ser Arg Leu Tyr Glu Glu Pro Ala Pro Asp
 515 520 525
 Arg Pro Pro Gly Glu Asn Glu Thr Tyr Leu Met Gln Phe Met Glu Glu
 530 535 540
 Trp Gly Leu Tyr Val Gln Met Glu Asn
 545 550

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<210> SEQ ID NO 21
<211> LENGTH: 911
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: BCAN

<400> SEQUENCE: 21

Met Ala Gln Leu Phe Leu Pro Leu Leu Ala Ala Leu Val Leu Ala Gln
1          5          10          15

Ala Pro Ala Ala Leu Ala Asp Val Leu Glu Gly Asp Ser Ser Glu Asp
          20          25          30

Arg Ala Phe Arg Val Arg Ile Ala Gly Asp Ala Pro Leu Gln Gly Val
          35          40          45

Leu Gly Gly Ala Leu Thr Ile Pro Cys His Val His Tyr Leu Arg Pro
50          55          60

Pro Pro Ser Arg Arg Ala Val Leu Gly Ser Pro Arg Val Lys Trp Thr
65          70          75          80

Phe Leu Ser Arg Gly Arg Glu Ala Glu Val Leu Val Ala Arg Gly Val
          85          90          95

Arg Val Lys Val Asn Glu Ala Tyr Arg Phe Arg Val Ala Leu Pro Ala
          100          105          110

Tyr Pro Ala Ser Leu Thr Asp Val Ser Leu Ala Leu Ser Glu Leu Arg
          115          120          125

Pro Asn Asp Ser Gly Ile Tyr Arg Cys Glu Val Gln His Gly Ile Asp
          130          135          140

Asp Ser Ser Asp Ala Val Glu Val Lys Val Lys Gly Val Val Phe Leu
          145          150          155          160

Tyr Arg Glu Gly Ser Ala Arg Tyr Ala Phe Ser Phe Ser Gly Ala Gln
          165          170          175

Glu Ala Cys Ala Arg Ile Gly Ala His Ile Ala Thr Pro Glu Gln Leu
          180          185          190

Tyr Ala Ala Tyr Leu Gly Gly Tyr Glu Gln Cys Asp Ala Gly Trp Leu
          195          200          205

Ser Asp Gln Thr Val Arg Tyr Pro Ile Gln Thr Pro Arg Glu Ala Cys
          210          215          220

Tyr Gly Asp Met Asp Gly Phe Pro Gly Val Arg Asn Tyr Gly Val Val
          225          230          235          240

Asp Pro Asp Asp Leu Tyr Asp Val Tyr Cys Tyr Ala Glu Asp Leu Asn
          245          250          255

Gly Glu Leu Phe Leu Gly Asp Pro Pro Glu Lys Leu Thr Leu Glu Glu
          260          265          270

Ala Arg Ala Tyr Cys Gln Glu Arg Gly Ala Glu Ile Ala Thr Thr Gly
          275          280          285

Gln Leu Tyr Ala Ala Trp Asp Gly Gly Leu Asp His Cys Ser Pro Gly
          290          295          300

Trp Leu Ala Asp Gly Ser Val Arg Tyr Pro Ile Val Thr Pro Ser Gln
          305          310          315          320

Arg Cys Gly Gly Gly Leu Pro Gly Val Lys Thr Leu Phe Leu Phe Pro
          325          330          335

Asn Gln Thr Gly Phe Pro Asn Lys His Ser Arg Phe Asn Val Tyr Cys
          340          345          350

Phe Arg Asp Ser Ala Gln Pro Ser Ala Ile Pro Glu Ala Ser Asn Pro
          355          360          365

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Ala Ser Asn Pro Ala Ser Asp Gly Leu Glu Ala Ile Val Thr Val Thr
 370 375 380
 Glu Thr Leu Glu Glu Leu Gln Leu Pro Gln Glu Ala Thr Glu Ser Glu
 385 390 395 400
 Ser Arg Gly Ala Ile Tyr Ser Ile Pro Ile Met Glu Asp Gly Gly Gly
 405 410 415
 Gly Ser Ser Thr Pro Glu Asp Pro Ala Glu Ala Pro Arg Thr Leu Leu
 420 425 430
 Glu Phe Glu Thr Gln Ser Met Val Pro Pro Thr Gly Phe Ser Glu Glu
 435 440 445
 Glu Gly Lys Ala Leu Glu Glu Glu Glu Lys Tyr Glu Asp Glu Glu Glu
 450 455 460
 Lys Glu Glu Glu Glu Glu Glu Glu Glu Val Glu Asp Glu Ala Leu Trp
 465 470 475 480
 Ala Trp Pro Ser Glu Leu Ser Ser Pro Gly Pro Glu Ala Ser Leu Pro
 485 490 495
 Thr Glu Pro Ala Ala Gln Glu Lys Ser Leu Ser Gln Ala Pro Ala Arg
 500 505 510
 Ala Val Leu Gln Pro Gly Ala Ser Pro Leu Pro Asp Gly Glu Ser Glu
 515 520 525
 Ala Ser Arg Pro Pro Arg Val His Gly Pro Pro Thr Glu Thr Leu Pro
 530 535 540
 Thr Pro Arg Glu Arg Asn Leu Ala Ser Pro Ser Pro Ser Thr Leu Val
 545 550 555 560
 Glu Ala Arg Glu Val Gly Glu Ala Thr Gly Gly Pro Glu Leu Ser Gly
 565 570 575
 Val Pro Arg Gly Glu Ser Glu Glu Thr Gly Ser Ser Glu Gly Ala Pro
 580 585 590
 Ser Leu Leu Pro Ala Thr Arg Ala Pro Glu Gly Thr Arg Glu Leu Glu
 595 600 605
 Ala Pro Ser Glu Asp Asn Ser Gly Arg Thr Ala Pro Ala Gly Thr Ser
 610 615 620
 Val Gln Ala Gln Pro Val Leu Pro Thr Asp Ser Ala Ser Arg Gly Gly
 625 630 635 640
 Val Ala Val Val Pro Ala Ser Gly Asp Cys Val Pro Ser Pro Cys His
 645 650 655
 Asn Gly Gly Thr Cys Leu Glu Glu Glu Glu Gly Val Arg Cys Leu Cys
 660 665 670
 Leu Pro Gly Tyr Gly Gly Asp Leu Cys Asp Val Gly Leu Arg Phe Cys
 675 680 685
 Asn Pro Gly Trp Asp Ala Phe Gln Gly Ala Cys Tyr Lys His Phe Ser
 690 695 700
 Thr Arg Arg Ser Trp Glu Glu Ala Glu Thr Gln Cys Arg Met Tyr Gly
 705 710 715 720
 Ala His Leu Ala Ser Ile Ser Thr Pro Glu Glu Gln Asp Phe Ile Asn
 725 730 735
 Asn Arg Tyr Arg Glu Tyr Gln Trp Ile Gly Leu Asn Asp Arg Thr Ile
 740 745 750
 Glu Gly Asp Phe Leu Trp Ser Asp Gly Val Pro Leu Leu Tyr Glu Asn
 755 760 765
 Trp Asn Pro Gly Gln Pro Asp Ser Tyr Phe Leu Ser Gly Glu Asn Cys
 770 775 780
 Val Val Met Val Trp His Asp Gln Gly Gln Trp Ser Asp Val Pro Cys

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785	790	795	800
Asn Tyr His Leu Ser Tyr Thr Cys Lys Met Gly Leu Val Ser Cys Gly	805	810	815
Pro Pro Pro Glu Leu Pro Leu Ala Gln Val Phe Gly Arg Pro Arg Leu	820	825	830
Arg Tyr Glu Val Asp Thr Val Leu Arg Tyr Arg Cys Arg Glu Gly Leu	835	840	845
Ala Gln Arg Asn Leu Pro Leu Ile Arg Cys Gln Glu Asn Gly Arg Trp	850	855	860
Glu Ala Pro Gln Ile Ser Cys Val Pro Arg Arg Pro Ala Arg Ala Leu	865	870	875
His Pro Glu Glu Asp Pro Glu Gly Arg Gln Gly Arg Leu Leu Gly Arg	885	890	895
Trp Lys Ala Leu Leu Ile Pro Pro Ser Ser Pro Met Pro Gly Pro	900	905	910

<210> SEQ ID NO 22
 <211> LENGTH: 987
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <223> OTHER INFORMATION: EphB2R

<400> SEQUENCE: 22

Met Ala Leu Arg Arg Leu Gly Ala Ala Leu Leu Leu Leu Pro Leu Leu	1	5	10	15
Ala Ala Val Glu Glu Thr Leu Met Asp Ser Thr Thr Ala Thr Ala Glu	20	25	30	
Leu Gly Trp Met Val His Pro Pro Ser Gly Trp Glu Glu Val Ser Gly	35	40	45	
Tyr Asp Glu Asn Met Asn Thr Ile Arg Thr Tyr Gln Val Cys Asn Val	50	55	60	
Phe Glu Ser Ser Gln Asn Asn Trp Leu Arg Thr Lys Phe Ile Arg Arg	65	70	75	80
Arg Gly Ala His Arg Ile His Val Glu Met Lys Phe Ser Val Arg Asp	85	90	95	
Cys Ser Ser Ile Pro Ser Val Pro Gly Ser Cys Lys Glu Thr Phe Asn	100	105	110	
Leu Tyr Tyr Tyr Glu Ala Asp Phe Asp Ser Ala Thr Lys Thr Phe Pro	115	120	125	
Asn Trp Met Glu Asn Pro Trp Val Lys Val Asp Thr Ile Ala Ala Asp	130	135	140	
Glu Ser Phe Ser Gln Val Asp Leu Gly Gly Arg Val Met Lys Ile Asn	145	150	155	160
Thr Glu Val Arg Ser Phe Gly Pro Val Ser Arg Ser Gly Phe Tyr Leu	165	170	175	
Ala Phe Gln Asp Tyr Gly Gly Cys Met Ser Leu Ile Ala Val Arg Val	180	185	190	
Phe Tyr Arg Lys Cys Pro Arg Ile Ile Gln Asn Gly Ala Ile Phe Gln	195	200	205	
Glu Thr Leu Ser Gly Ala Glu Ser Thr Ser Leu Val Ala Ala Arg Gly	210	215	220	
Ser Cys Ile Ala Asn Ala Glu Glu Val Asp Val Pro Ile Lys Leu Tyr	225	230	235	240
Cys Asn Gly Asp Gly Glu Trp Leu Val Pro Ile Gly Arg Cys Met Cys				

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245					250					255					
Lys	Ala	Gly	Phe	Glu	Ala	Val	Glu	Asn	Gly	Thr	Val	Cys	Arg	Gly	Cys
			260					265					270		
Pro	Ser	Gly	Thr	Phe	Lys	Ala	Asn	Gln	Gly	Asp	Glu	Ala	Cys	Thr	His
		275					280					285			
Cys	Pro	Ile	Asn	Ser	Arg	Thr	Thr	Ser	Glu	Gly	Ala	Thr	Asn	Cys	Val
	290					295					300				
Cys	Arg	Asn	Gly	Tyr	Tyr	Arg	Ala	Asp	Leu	Asp	Pro	Leu	Asp	Met	Pro
305				310					315					320	
Cys	Thr	Thr	Ile	Pro	Ser	Ala	Pro	Gln	Ala	Val	Ile	Ser	Ser	Val	Asn
			325						330					335	
Glu	Thr	Ser	Leu	Met	Leu	Glu	Trp	Thr	Pro	Pro	Arg	Asp	Ser	Gly	Gly
			340					345					350		
Arg	Glu	Asp	Leu	Val	Tyr	Asn	Ile	Ile	Cys	Lys	Ser	Cys	Gly	Ser	Gly
		355					360					365			
Arg	Gly	Ala	Cys	Thr	Arg	Cys	Gly	Asp	Asn	Val	Gln	Tyr	Ala	Pro	Arg
	370					375						380			
Gln	Leu	Gly	Leu	Thr	Glu	Pro	Arg	Ile	Tyr	Ile	Ser	Asp	Leu	Leu	Ala
385						390					395				400
His	Thr	Gln	Tyr	Thr	Phe	Glu	Ile	Gln	Ala	Val	Asn	Gly	Val	Thr	Asp
			405					410						415	
Gln	Ser	Pro	Phe	Ser	Pro	Gln	Phe	Ala	Ser	Val	Asn	Ile	Thr	Thr	Asn
			420				425						430		
Gln	Ala	Ala	Pro	Ser	Ala	Val	Ser	Ile	Met	His	Gln	Val	Ser	Arg	Thr
	435						440					445			
Val	Asp	Ser	Ile	Thr	Leu	Ser	Trp	Ser	Gln	Pro	Asp	Gln	Pro	Asn	Gly
	450					455					460				
Val	Ile	Leu	Asp	Tyr	Glu	Leu	Gln	Tyr	Tyr	Glu	Lys	Glu	Leu	Ser	Glu
465				470					475						480
Tyr	Asn	Ala	Thr	Ala	Ile	Lys	Ser	Pro	Thr	Asn	Thr	Val	Thr	Val	Gln
			485						490					495	
Gly	Leu	Lys	Ala	Gly	Ala	Ile	Tyr	Val	Phe	Gln	Val	Arg	Ala	Arg	Thr
			500					505				510			
Val	Ala	Gly	Tyr	Gly	Arg	Tyr	Ser	Gly	Lys	Met	Tyr	Phe	Gln	Thr	Met
	515						520					525			
Thr	Glu	Ala	Glu	Tyr	Gln	Thr	Ser	Ile	Gln	Glu	Lys	Leu	Pro	Leu	Ile
	530					535					540				
Ile	Gly	Ser	Ser	Ala	Ala	Gly	Leu	Val	Phe	Leu	Ile	Ala	Val	Val	Val
545				550							555				560
Ile	Ala	Ile	Val	Cys	Asn	Arg	Arg	Arg	Gly	Phe	Glu	Arg	Ala	Asp	Ser
			565						570					575	
Glu	Tyr	Thr	Asp	Lys	Leu	Gln	His	Tyr	Thr	Ser	Gly	His	Met	Thr	Pro
			580				585						590		
Gly	Met	Lys	Ile	Tyr	Ile	Asp	Pro	Phe	Thr	Tyr	Glu	Asp	Pro	Asn	Glu
		595					600					605			
Ala	Val	Arg	Glu	Phe	Ala	Lys	Glu	Ile	Asp	Ile	Ser	Cys	Val	Lys	Ile
	610					615					620				
Glu	Gln	Val	Ile	Gly	Ala	Gly	Glu	Phe	Gly	Glu	Val	Cys	Ser	Gly	His
625				630							635				640
Leu	Lys	Leu	Pro	Gly	Lys	Arg	Glu	Ile	Phe	Val	Ala	Ile	Lys	Thr	Leu
			645					650						655	
Lys	Ser	Gly	Tyr	Thr	Glu	Lys	Gln	Arg	Arg	Asp	Phe	Leu	Ser	Glu	Ala
			660				665						670		

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Ser Ile Met Gly Gln Phe Asp His Pro Asn Val Ile His Leu Glu Gly
 675 680 685
 Val Val Thr Lys Ser Thr Pro Val Met Ile Ile Thr Glu Phe Met Glu
 690 695 700
 Asn Gly Ser Leu Asp Ser Phe Leu Arg Gln Asn Asp Gly Gln Phe Thr
 705 710 715 720
 Val Ile Gln Leu Val Gly Met Leu Arg Gly Ile Ala Ala Gly Met Lys
 725 730 735
 Tyr Leu Ala Asp Met Asn Tyr Val His Arg Asp Leu Ala Ala Arg Asn
 740 745 750
 Ile Leu Val Asn Ser Asn Leu Val Cys Lys Val Ser Asp Phe Gly Leu
 755 760 765
 Ser Arg Phe Leu Glu Asp Asp Thr Ser Asp Pro Thr Tyr Thr Ser Ala
 770 775 780
 Leu Gly Gly Lys Ile Pro Ile Arg Trp Thr Ala Pro Glu Ala Ile Gln
 785 790 795 800
 Tyr Arg Lys Phe Thr Ser Ala Ser Asp Val Trp Ser Tyr Gly Ile Val
 805 810 815
 Met Trp Glu Val Met Ser Tyr Gly Glu Arg Pro Tyr Trp Asp Met Thr
 820 825 830
 Asn Gln Asp Val Ile Asn Ala Ile Glu Gln Asp Tyr Arg Leu Pro Pro
 835 840 845
 Pro Met Asp Cys Pro Ser Ala Leu His Gln Leu Met Leu Asp Cys Trp
 850 855 860
 Gln Lys Asp Arg Asn His Arg Pro Lys Phe Gly Gln Ile Val Asn Thr
 865 870 875 880
 Leu Asp Lys Met Ile Arg Asn Pro Asn Ser Leu Lys Ala Met Ala Pro
 885 890 895
 Leu Ser Ser Gly Ile Asn Leu Pro Leu Leu Asp Arg Thr Ile Pro Asp
 900 905 910
 Tyr Thr Ser Phe Asn Thr Val Asp Glu Trp Leu Glu Ala Ile Lys Met
 915 920 925
 Gly Gln Tyr Lys Glu Ser Phe Ala Asn Ala Gly Phe Thr Ser Phe Asp
 930 935 940
 Val Val Ser Gln Met Met Met Glu Asp Ile Leu Arg Val Gly Val Thr
 945 950 955 960
 Leu Ala Gly His Gln Lys Lys Ile Leu Asn Ser Ile Gln Val Met Arg
 965 970 975
 Ala Gln Met Asn Gln Ile Gln Ser Val Glu Val
 980 985

<210> SEQ ID NO 23
 <211> LENGTH: 282
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <223> OTHER INFORMATION: ASLG659

<400> SEQUENCE: 23

Met Ala Ser Leu Gly Gln Ile Leu Phe Trp Ser Ile Ile Ser Ile Ile
 1 5 10 15
 Ile Ile Leu Ala Gly Ala Ile Ala Leu Ile Ile Gly Phe Gly Ile Ser
 20 25 30
 Gly Arg His Ser Ile Thr Val Thr Thr Val Ala Ser Ala Gly Asn Ile
 35 40 45

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Gly Glu Asp Gly Ile Leu Ser Cys Thr Phe Glu Pro Asp Ile Lys Leu
 50 55 60
 Ser Asp Ile Val Ile Gln Trp Leu Lys Glu Gly Val Leu Gly Leu Val
 65 70 75 80
 His Glu Phe Lys Glu Gly Lys Asp Glu Leu Ser Glu Gln Asp Glu Met
 85 90 95
 Phe Arg Gly Arg Thr Ala Val Phe Ala Asp Gln Val Ile Val Gly Asn
 100 105 110
 Ala Ser Leu Arg Leu Lys Asn Val Gln Leu Thr Asp Ala Gly Thr Tyr
 115 120 125
 Lys Cys Tyr Ile Ile Thr Ser Lys Gly Lys Lys Asn Ala Asn Leu Glu
 130 135 140
 Tyr Lys Thr Gly Ala Phe Ser Met Pro Glu Val Asn Val Asp Tyr Asn
 145 150 155 160
 Ala Ser Ser Glu Thr Leu Arg Cys Glu Ala Pro Arg Trp Phe Pro Gln
 165 170 175
 Pro Thr Val Val Trp Ala Ser Gln Val Asp Gln Gly Ala Asn Phe Ser
 180 185 190
 Glu Val Ser Asn Thr Ser Phe Glu Leu Asn Ser Glu Asn Val Thr Met
 195 200 205
 Lys Val Val Ser Val Leu Tyr Asn Val Thr Ile Asn Asn Thr Tyr Ser
 210 215 220
 Cys Met Ile Glu Asn Asp Ile Ala Lys Ala Thr Gly Asp Ile Lys Val
 225 230 235 240
 Thr Glu Ser Glu Ile Lys Arg Arg Ser His Leu Gln Leu Leu Asn Ser
 245 250 255
 Lys Ala Ser Leu Cys Val Ser Ser Phe Phe Ala Ile Ser Trp Ala Leu
 260 265 270
 Leu Pro Leu Ser Pro Tyr Leu Met Leu Lys
 275 280

<210> SEQ ID NO 24
 <211> LENGTH: 123
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <223> OTHER INFORMATION: PSCA

<400> SEQUENCE: 24

Met Lys Ala Val Leu Leu Ala Leu Leu Met Ala Gly Leu Ala Leu Gln
 1 5 10 15
 Pro Gly Thr Ala Leu Leu Cys Tyr Ser Cys Lys Ala Gln Val Ser Asn
 20 25 30
 Glu Asp Cys Leu Gln Val Glu Asn Cys Thr Gln Leu Gly Glu Gln Cys
 35 40 45
 Trp Thr Ala Arg Ile Arg Ala Val Gly Leu Leu Thr Val Ile Ser Lys
 50 55 60
 Gly Cys Ser Leu Asn Cys Val Asp Asp Ser Gln Asp Tyr Tyr Val Gly
 65 70 75 80
 Lys Lys Asn Ile Thr Cys Cys Asp Thr Asp Leu Cys Asn Ala Ser Gly
 85 90 95
 Ala His Ala Leu Gln Pro Ala Ala Ala Ile Leu Ala Leu Leu Pro Ala
 100 105 110
 Leu Gly Leu Leu Leu Trp Gly Pro Gly Gln Leu
 115 120

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<210> SEQ ID NO 25
 <211> LENGTH: 236
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <223> OTHER INFORMATION: GEDA

<400> SEQUENCE: 25

Met Pro Gly Ala Ala Ala Ala Ala Ala Ala Ala Ala Ala Met Leu
 1 5 10 15
 Pro Ala Gln Glu Ala Ala Lys Leu Tyr His Thr Asn Tyr Val Arg Asn
 20 25 30
 Ser Arg Ala Ile Gly Val Leu Trp Ala Ile Phe Thr Ile Cys Phe Ala
 35 40 45
 Ile Val Asn Val Val Cys Phe Ile Gln Pro Tyr Trp Ile Gly Asp Gly
 50 55 60
 Val Asp Thr Pro Gln Ala Gly Tyr Phe Gly Leu Phe His Tyr Cys Ile
 65 70 75 80
 Gly Asn Gly Phe Ser Arg Glu Leu Thr Cys Arg Gly Ser Phe Thr Asp
 85 90 95
 Phe Ser Thr Leu Pro Ser Gly Ala Phe Lys Ala Ala Ser Phe Phe Ile
 100 105 110
 Gly Leu Ser Met Met Leu Ile Ile Ala Cys Ile Ile Cys Phe Thr Leu
 115 120 125
 Phe Phe Phe Cys Asn Thr Ala Thr Val Tyr Lys Ile Cys Ala Trp Met
 130 135 140
 Gln Leu Thr Ser Ala Ala Cys Leu Val Leu Gly Cys Met Ile Phe Pro
 145 150 155 160
 Asp Gly Trp Asp Ser Asp Glu Val Lys Arg Met Cys Gly Glu Lys Thr
 165 170 175
 Asp Lys Tyr Thr Leu Gly Ala Cys Ser Val Arg Trp Ala Tyr Ile Leu
 180 185 190
 Ala Ile Ile Gly Ile Leu Asp Ala Leu Ile Leu Ser Phe Leu Ala Phe
 195 200 205
 Val Leu Gly Asn Arg Gln Asp Ser Leu Met Ala Glu Glu Leu Lys Ala
 210 215 220
 Glu Asn Lys Val Leu Leu Ser Gln Tyr Ser Leu Glu
 225 230 235

<210> SEQ ID NO 26
 <211> LENGTH: 184
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <223> OTHER INFORMATION: BAPF-R

<400> SEQUENCE: 26

Met Arg Arg Gly Pro Arg Ser Leu Arg Gly Arg Asp Ala Pro Ala Pro
 1 5 10 15
 Thr Pro Cys Val Pro Ala Glu Cys Phe Asp Leu Leu Val Arg His Cys
 20 25 30
 Val Ala Cys Gly Leu Leu Arg Thr Pro Arg Pro Lys Pro Ala Gly Ala
 35 40 45
 Ser Ser Pro Ala Pro Arg Thr Ala Leu Gln Pro Gln Glu Ser Val Gly
 50 55 60
 Ala Gly Ala Gly Glu Ala Ala Leu Pro Leu Pro Gly Leu Leu Phe Gly

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65	70	75	80
Ala Pro Ala Leu Leu Gly Leu Ala Leu Val Leu Ala Leu Val Leu Val			
	85	90	95
Gly Leu Val Ser Trp Arg Arg Arg Gln Arg Arg Leu Arg Gly Ala Ser			
	100	105	110
Ser Ala Glu Ala Pro Asp Gly Asp Lys Asp Ala Pro Glu Pro Leu Asp			
	115	120	125
Lys Val Ile Ile Leu Ser Pro Gly Ile Ser Asp Ala Thr Ala Pro Ala			
	130	135	140
Trp Pro Pro Pro Gly Glu Asp Pro Gly Thr Thr Pro Pro Gly His Ser			
	145	150	155
Val Pro Val Pro Ala Thr Glu Leu Gly Ser Thr Glu Leu Val Thr Thr			
	165	170	175
Lys Thr Ala Gly Pro Glu Gln Gln			
	180		

<210> SEQ ID NO 27
 <211> LENGTH: 847
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <223> OTHER INFORMATION: CD22

<400> SEQUENCE: 27

Met His Leu Leu Gly Pro Trp Leu Leu Leu Leu Val Leu Glu Tyr Leu			
1	5	10	15
Ala Phe Ser Asp Ser Ser Lys Trp Val Phe Glu His Pro Glu Thr Leu			
	20	25	30
Tyr Ala Trp Glu Gly Ala Cys Val Trp Ile Pro Cys Thr Tyr Arg Ala			
	35	40	45
Leu Asp Gly Asp Leu Glu Ser Phe Ile Leu Phe His Asn Pro Glu Tyr			
	50	55	60
Asn Lys Asn Thr Ser Lys Phe Asp Gly Thr Arg Leu Tyr Glu Ser Thr			
65	70	75	80
Lys Asp Gly Lys Val Pro Ser Glu Gln Lys Arg Val Gln Phe Leu Gly			
	85	90	95
Asp Lys Asn Lys Asn Cys Thr Leu Ser Ile His Pro Val His Leu Asn			
	100	105	110
Asp Ser Gly Gln Leu Gly Leu Arg Met Glu Ser Lys Thr Glu Lys Trp			
	115	120	125
Met Glu Arg Ile His Leu Asn Val Ser Glu Arg Pro Phe Pro Pro His			
	130	135	140
Ile Gln Leu Pro Pro Glu Ile Gln Glu Ser Gln Glu Val Thr Leu Thr			
	145	150	155
Cys Leu Leu Asn Phe Ser Cys Tyr Gly Tyr Pro Ile Gln Leu Gln Trp			
	165	170	175
Leu Leu Glu Gly Val Pro Met Arg Gln Ala Ala Val Thr Ser Thr Ser			
	180	185	190
Leu Thr Ile Lys Ser Val Phe Thr Arg Ser Glu Leu Lys Phe Ser Pro			
	195	200	205
Gln Trp Ser His His Gly Lys Ile Val Thr Cys Gln Leu Gln Asp Ala			
	210	215	220
Asp Gly Lys Phe Leu Ser Asn Asp Thr Val Gln Leu Asn Val Lys His			
	225	230	235
Thr Pro Lys Leu Glu Ile Lys Val Thr Pro Ser Asp Ala Ile Val Arg			

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245							250				255				
Glu	Gly	Asp	Ser	Val	Thr	Met	Thr	Cys	Glu	Val	Ser	Ser	Ser	Asn	Pro
			260					265					270		
Glu	Tyr	Thr	Thr	Val	Ser	Trp	Leu	Lys	Asp	Gly	Thr	Ser	Leu	Lys	Lys
		275					280					285			
Gln	Asn	Thr	Phe	Thr	Leu	Asn	Leu	Arg	Glu	Val	Thr	Lys	Asp	Gln	Ser
		290				295					300				
Gly	Lys	Tyr	Cys	Cys	Gln	Val	Ser	Asn	Asp	Val	Gly	Pro	Gly	Arg	Ser
305					310					315				320	
Glu	Glu	Val	Phe	Leu	Gln	Val	Gln	Tyr	Ala	Pro	Glu	Pro	Ser	Thr	Val
			325						330					335	
Gln	Ile	Leu	His	Ser	Pro	Ala	Val	Glu	Gly	Ser	Gln	Val	Glu	Phe	Leu
		340						345					350		
Cys	Met	Ser	Leu	Ala	Asn	Pro	Leu	Pro	Thr	Asn	Tyr	Thr	Trp	Tyr	His
		355					360					365			
Asn	Gly	Lys	Glu	Met	Gln	Gly	Arg	Thr	Glu	Glu	Lys	Val	His	Ile	Pro
	370					375					380				
Lys	Ile	Leu	Pro	Trp	His	Ala	Gly	Thr	Tyr	Ser	Cys	Val	Ala	Glu	Asn
385					390					395				400	
Ile	Leu	Gly	Thr	Gly	Gln	Arg	Gly	Pro	Gly	Ala	Glu	Leu	Asp	Val	Gln
				405					410					415	
Tyr	Pro	Pro	Lys	Lys	Val	Thr	Thr	Val	Ile	Gln	Asn	Pro	Met	Pro	Ile
			420					425					430		
Arg	Glu	Gly	Asp	Thr	Val	Thr	Leu	Ser	Cys	Asn	Tyr	Asn	Ser	Ser	Asn
		435					440					445			
Pro	Ser	Val	Thr	Arg	Tyr	Glu	Trp	Lys	Pro	His	Gly	Ala	Trp	Glu	Glu
	450					455					460				
Pro	Ser	Leu	Gly	Val	Leu	Lys	Ile	Gln	Asn	Val	Gly	Trp	Asp	Asn	Thr
465				470						475				480	
Thr	Ile	Ala	Cys	Ala	Arg	Cys	Asn	Ser	Trp	Cys	Ser	Trp	Ala	Ser	Pro
			485						490					495	
Val	Ala	Leu	Asn	Val	Gln	Tyr	Ala	Pro	Arg	Asp	Val	Arg	Val	Arg	Lys
		500						505					510		
Ile	Lys	Pro	Leu	Ser	Glu	Ile	His	Ser	Gly	Asn	Ser	Val	Ser	Leu	Gln
		515				520						525			
Cys	Asp	Phe	Ser	Ser	Ser	His	Pro	Lys	Glu	Val	Gln	Phe	Phe	Trp	Glu
	530					535					540				
Lys	Asn	Gly	Arg	Leu	Leu	Gly	Lys	Glu	Ser	Gln	Leu	Asn	Phe	Asp	Ser
545				550						555				560	
Ile	Ser	Pro	Glu	Asp	Ala	Gly	Ser	Tyr	Ser	Cys	Trp	Val	Asn	Asn	Ser
			565						570				575		
Ile	Gly	Gln	Thr	Ala	Ser	Lys	Ala	Trp	Thr	Leu	Glu	Val	Leu	Tyr	Ala
		580						585					590		
Pro	Arg	Arg	Leu	Arg	Val	Ser	Met	Ser	Pro	Gly	Asp	Gln	Val	Met	Glu
		595					600					605			
Gly	Lys	Ser	Ala	Thr	Leu	Thr	Cys	Glu	Ser	Asp	Ala	Asn	Pro	Pro	Val
	610					615					620				
Ser	His	Tyr	Thr	Trp	Phe	Asp	Trp	Asn	Asn	Gln	Ser	Leu	Pro	His	His
625					630					635				640	
Ser	Gln	Lys	Leu	Arg	Leu	Glu	Pro	Val	Lys	Val	Gln	His	Ser	Gly	Ala
			645						650				655		
Tyr	Trp	Cys	Gln	Gly	Thr	Asn	Ser	Val	Gly	Lys	Gly	Arg	Ser	Pro	Leu
		660						665					670		

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Ser Thr Leu Thr Val Tyr Tyr Ser Pro Glu Thr Ile Gly Arg Arg Val
675 680 685

Ala Val Gly Leu Gly Ser Cys Leu Ala Ile Leu Ile Leu Ala Ile Cys
690 695 700

Gly Leu Lys Leu Gln Arg Arg Trp Lys Arg Thr Gln Ser Gln Gln Gly
705 710 715 720

Leu Gln Glu Asn Ser Ser Gly Gln Ser Phe Phe Val Arg Asn Lys Lys
725 730 735

Val Arg Arg Ala Pro Leu Ser Glu Gly Pro His Ser Leu Gly Cys Tyr
740 745 750

Asn Pro Met Met Glu Asp Gly Ile Ser Tyr Thr Thr Leu Arg Phe Pro
755 760 765

Glu Met Asn Ile Pro Arg Thr Gly Asp Ala Glu Ser Glu Met Gln
770 775 780

Arg Pro Pro Arg Thr Cys Asp Asp Thr Val Thr Tyr Ser Ala Leu His
785 790 795 800

Lys Arg Gln Val Gly Asp Tyr Glu Asn Val Ile Pro Asp Phe Pro Glu
805 810 815

Asp Glu Gly Ile His Tyr Ser Glu Leu Ile Gln Phe Gly Val Gly Glu
820 825 830

Arg Pro Gln Ala Gln Glu Asn Val Asp Tyr Val Ile Leu Lys His
835 840 845

<210> SEQ ID NO 28
 <211> LENGTH: 226
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <223> OTHER INFORMATION: CD79A

<400> SEQUENCE: 28

Met Pro Gly Gly Pro Gly Val Leu Gln Ala Leu Pro Ala Thr Ile Phe
1 5 10 15

Leu Leu Phe Leu Leu Ser Ala Val Tyr Leu Gly Pro Gly Cys Gln Ala
20 25 30

Leu Trp Met His Lys Val Pro Ala Ser Leu Met Val Ser Leu Gly Glu
35 40 45

Asp Ala His Phe Gln Cys Pro His Asn Ser Ser Asn Asn Ala Asn Val
50 55 60

Thr Trp Trp Arg Val Leu His Gly Asn Tyr Thr Trp Pro Pro Glu Phe
65 70 75 80

Leu Gly Pro Gly Glu Asp Pro Asn Gly Thr Leu Ile Ile Gln Asn Val
85 90 95

Asn Lys Ser His Gly Gly Ile Tyr Val Cys Arg Val Gln Glu Gly Asn
100 105 110

Glu Ser Tyr Gln Gln Ser Cys Gly Thr Tyr Leu Arg Val Arg Gln Pro
115 120 125

Pro Pro Arg Pro Phe Leu Asp Met Gly Glu Gly Thr Lys Asn Arg Ile
130 135 140

Ile Thr Ala Glu Gly Ile Ile Leu Leu Phe Cys Ala Val Val Pro Gly
145 150 155 160

Thr Leu Leu Leu Phe Arg Lys Arg Trp Gln Asn Glu Lys Leu Gly Leu
165 170 175

Asp Ala Gly Asp Glu Tyr Glu Asp Glu Asn Leu Tyr Glu Gly Leu Asn
180 185 190

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Leu Asp Asp Cys Ser Met Tyr Glu Asp Ile Ser Arg Gly Leu Gln Gly
195 200 205

Thr Tyr Gln Asp Val Gly Ser Leu Asn Ile Gly Asp Val Gln Leu Glu
210 215 220

Lys Pro
225

<210> SEQ ID NO 29
<211> LENGTH: 372
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: CXCR5

<400> SEQUENCE: 29

Met Asn Tyr Pro Leu Thr Leu Glu Met Asp Leu Glu Asn Leu Glu Asp
1 5 10 15

Leu Phe Trp Glu Leu Asp Arg Leu Asp Asn Tyr Asn Asp Thr Ser Leu
20 25 30

Val Glu Asn His Leu Cys Pro Ala Thr Glu Gly Pro Leu Met Ala Ser
35 40 45

Phe Lys Ala Val Phe Val Pro Val Ala Tyr Ser Leu Ile Phe Leu Leu
50 55 60

Gly Val Ile Gly Asn Val Leu Val Leu Val Ile Leu Glu Arg His Arg
65 70 75 80

Gln Thr Arg Ser Ser Thr Glu Thr Phe Leu Phe His Leu Ala Val Ala
85 90 95

Asp Leu Leu Leu Val Phe Ile Leu Pro Phe Ala Val Ala Glu Gly Ser
100 105 110

Val Gly Trp Val Leu Gly Thr Phe Leu Cys Lys Thr Val Ile Ala Leu
115 120 125

His Lys Val Asn Phe Tyr Cys Ser Ser Leu Leu Leu Ala Cys Ile Ala
130 135 140

Val Asp Arg Tyr Leu Ala Ile Val His Ala Val His Ala Tyr Arg His
145 150 155 160

Arg Arg Leu Leu Ser Ile His Ile Thr Cys Gly Thr Ile Trp Leu Val
165 170 175

Gly Phe Leu Leu Ala Leu Pro Glu Ile Leu Phe Ala Lys Val Ser Gln
180 185 190

Gly His His Asn Asn Ser Leu Pro Arg Cys Thr Phe Ser Gln Glu Asn
195 200 205

Gln Ala Glu Thr His Ala Trp Phe Thr Ser Arg Phe Leu Tyr His Val
210 215 220

Ala Gly Phe Leu Leu Pro Met Leu Val Met Gly Trp Cys Tyr Val Gly
225 230 235 240

Val Val His Arg Leu Arg Gln Ala Gln Arg Arg Pro Gln Arg Gln Lys
245 250 255

Ala Val Arg Val Ala Ile Leu Val Thr Ser Ile Phe Phe Leu Cys Trp
260 265 270

Ser Pro Tyr His Ile Val Ile Phe Leu Asp Thr Leu Ala Arg Leu Lys
275 280 285

Ala Val Asp Asn Thr Cys Lys Leu Asn Gly Ser Leu Pro Val Ala Ile
290 295 300

Thr Met Cys Glu Phe Leu Gly Leu Ala His Cys Cys Leu Asn Pro Met
305 310 315 320

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Leu Tyr Thr Phe Ala Gly Val Lys Phe Arg Ser Asp Leu Ser Arg Leu
 325 330 335

Leu Thr Lys Leu Gly Cys Thr Gly Pro Ala Ser Leu Cys Gln Leu Phe
 340 345 350

Pro Ser Trp Arg Arg Ser Ser Leu Ser Glu Ser Glu Asn Ala Thr Ser
 355 360 365

Leu Thr Thr Phe
 370

<210> SEQ ID NO 30
 <211> LENGTH: 273
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <223> OTHER INFORMATION: HLA-DOB

<400> SEQUENCE: 30

Met Gly Ser Gly Trp Val Pro Trp Val Val Ala Leu Leu Val Asn Leu
 1 5 10 15

Thr Arg Leu Asp Ser Ser Met Thr Gln Gly Thr Asp Ser Pro Glu Asp
 20 25 30

Phe Val Ile Gln Ala Lys Ala Asp Cys Tyr Phe Thr Asn Gly Thr Glu
 35 40 45

Lys Val Gln Phe Val Val Arg Phe Ile Phe Asn Leu Glu Glu Tyr Val
 50 55 60

Arg Phe Asp Ser Asp Val Gly Met Phe Val Ala Leu Thr Lys Leu Gly
 65 70 75 80

Gln Pro Asp Ala Glu Gln Trp Asn Ser Arg Leu Asp Leu Leu Glu Arg
 85 90 95

Ser Arg Gln Ala Val Asp Gly Val Cys Arg His Asn Tyr Arg Leu Gly
 100 105 110

Ala Pro Phe Thr Val Gly Arg Lys Val Gln Pro Glu Val Thr Val Tyr
 115 120 125

Pro Glu Arg Thr Pro Leu Leu His Gln His Asn Leu Leu His Cys Ser
 130 135 140

Val Thr Gly Phe Tyr Pro Gly Asp Ile Lys Ile Lys Trp Phe Leu Asn
 145 150 155 160

Gly Gln Glu Glu Arg Ala Gly Val Met Ser Thr Gly Pro Ile Arg Asn
 165 170 175

Gly Asp Trp Thr Phe Gln Thr Val Val Met Leu Glu Met Thr Pro Glu
 180 185 190

Leu Gly His Val Tyr Thr Cys Leu Val Asp His Ser Ser Leu Leu Ser
 195 200 205

Pro Val Ser Val Glu Trp Arg Ala Gln Ser Glu Tyr Ser Trp Arg Lys
 210 215 220

Met Leu Ser Gly Ile Ala Ala Phe Leu Leu Gly Leu Ile Phe Leu Leu
 225 230 235 240

Val Gly Ile Val Ile Gln Leu Arg Ala Gln Lys Gly Tyr Val Arg Thr
 245 250 255

Gln Met Ser Gly Asn Glu Val Ser Arg Ala Val Leu Leu Pro Gln Ser
 260 265 270

Cys

<210> SEQ ID NO 31
 <211> LENGTH: 422

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<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: Purinergic receptor P2X ligand-gated ion
channel 5

<400> SEQUENCE: 31
Met Gly Gln Ala Gly Cys Lys Gly Leu Cys Leu Ser Leu Phe Asp Tyr
1          5          10          15
Lys Thr Glu Lys Tyr Val Ile Ala Lys Asn Lys Lys Val Gly Leu Leu
20          25          30
Tyr Arg Leu Leu Gln Ala Ser Ile Leu Ala Tyr Leu Val Val Trp Val
35          40          45
Phe Leu Ile Lys Lys Gly Tyr Gln Asp Val Asp Thr Ser Leu Gln Ser
50          55          60
Ala Val Ile Thr Lys Val Lys Gly Val Ala Phe Thr Asn Thr Ser Asp
65          70          75          80
Leu Gly Gln Arg Ile Trp Asp Val Ala Asp Tyr Val Ile Pro Ala Gln
85          90          95
Gly Glu Asn Val Phe Phe Val Val Thr Asn Leu Ile Val Thr Pro Asn
100         105         110
Gln Arg Gln Asn Val Cys Ala Glu Asn Glu Gly Ile Pro Asp Gly Ala
115         120         125
Cys Ser Lys Asp Ser Asp Cys His Ala Gly Glu Ala Val Thr Ala Gly
130         135         140
Asn Gly Val Lys Thr Gly Arg Cys Leu Arg Arg Glu Asn Leu Ala Arg
145         150         155         160
Gly Thr Cys Glu Ile Phe Ala Trp Cys Pro Leu Glu Thr Ser Ser Arg
165         170         175
Pro Glu Glu Pro Phe Leu Lys Glu Ala Glu Asp Phe Thr Ile Phe Ile
180         185         190
Lys Asn His Ile Arg Phe Pro Lys Phe Asn Phe Ser Lys Ser Asn Val
195         200         205
Met Asp Val Lys Asp Arg Ser Phe Leu Lys Ser Cys His Phe Gly Pro
210         215         220
Lys Asn His Tyr Cys Pro Ile Phe Arg Leu Gly Ser Val Ile Arg Trp
225         230         235         240
Ala Gly Ser Asp Phe Gln Asp Ile Ala Leu Glu Gly Gly Val Ile Gly
245         250         255
Ile Asn Ile Glu Trp Asn Cys Asp Leu Asp Lys Ala Ala Ser Glu Cys
260         265         270
His Pro His Tyr Ser Phe Ser Arg Leu Asp Asn Lys Leu Ser Lys Ser
275         280         285
Val Ser Ser Gly Tyr Asn Phe Arg Phe Ala Arg Tyr Tyr Arg Asp Ala
290         295         300
Ala Gly Val Glu Phe Arg Thr Leu Met Lys Ala Tyr Gly Ile Arg Phe
305         310         315         320
Asp Val Met Val Asn Gly Lys Gly Ala Phe Phe Cys Asp Leu Val Leu
325         330         335
Ile Tyr Leu Ile Lys Lys Arg Glu Phe Tyr Arg Asp Lys Lys Tyr Glu
340         345         350
Glu Val Arg Gly Leu Glu Asp Ser Ser Gln Glu Ala Glu Asp Glu Ala
355         360         365
Ser Gly Leu Gly Leu Ser Glu Gln Leu Thr Ser Gly Pro Gly Leu Leu
370         375         380

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Gly Met Pro Glu Gln Gln Glu Leu Gln Glu Pro Pro Glu Ala Lys Arg
385 390 395 400

Gly Ser Ser Ser Gln Lys Gly Asn Gly Ser Val Cys Pro Gln Leu Leu
405 410 415

Glu Pro His Arg Ser Thr
420

<210> SEQ ID NO 32

<211> LENGTH: 359

<212> TYPE: PRT

<213> ORGANISM: Homo sapiens

<220> FEATURE:

<223> OTHER INFORMATION: CD72

<400> SEQUENCE: 32

Met Ala Glu Ala Ile Thr Tyr Ala Asp Leu Arg Phe Val Lys Ala Pro
1 5 10 15

Leu Lys Lys Ser Ile Ser Ser Arg Leu Gly Gln Asp Pro Gly Ala Asp
20 25 30

Asp Asp Gly Glu Ile Thr Tyr Glu Asn Val Gln Val Pro Ala Val Leu
35 40 45

Gly Val Pro Ser Ser Leu Ala Ser Ser Val Leu Gly Asp Lys Ala Ala
50 55 60

Val Lys Ser Glu Gln Pro Thr Ala Ser Trp Arg Ala Val Thr Ser Pro
65 70 75 80

Ala Val Gly Arg Ile Leu Pro Cys Arg Thr Thr Cys Leu Arg Tyr Leu
85 90 95

Leu Leu Gly Leu Leu Leu Thr Cys Leu Leu Gly Val Thr Ala Ile
100 105 110

Cys Leu Gly Val Arg Tyr Leu Gln Val Ser Gln Gln Leu Gln Gln Thr
115 120 125

Asn Arg Val Leu Glu Val Thr Asn Ser Ser Leu Arg Gln Gln Leu Arg
130 135 140

Leu Lys Ile Thr Gln Leu Gly Gln Ser Ala Glu Asp Leu Gln Gly Ser
145 150 155 160

Arg Arg Glu Leu Ala Gln Ser Gln Glu Ala Leu Gln Val Glu Gln Arg
165 170 175

Ala His Gln Ala Ala Glu Gly Gln Leu Gln Ala Cys Gln Ala Asp Arg
180 185 190

Gln Lys Thr Lys Glu Thr Leu Gln Ser Glu Glu Gln Gln Arg Arg Ala
195 200 205

Leu Glu Gln Lys Leu Ser Asn Met Glu Asn Arg Leu Lys Pro Phe Phe
210 215 220

Thr Cys Gly Ser Ala Asp Thr Cys Cys Pro Ser Gly Trp Ile Met His
225 230 235 240

Gln Lys Ser Cys Phe Tyr Ile Ser Leu Thr Ser Lys Asn Trp Gln Glu
245 250 255

Ser Gln Lys Gln Cys Glu Thr Leu Ser Ser Lys Leu Ala Thr Phe Ser
260 265 270

Glu Ile Tyr Pro Gln Ser His Ser Tyr Tyr Phe Leu Asn Ser Leu Leu
275 280 285

Pro Asn Gly Gly Ser Gly Asn Ser Tyr Trp Thr Gly Leu Ser Ser Asn
290 295 300

Lys Asp Trp Lys Leu Thr Asp Asp Thr Gln Arg Thr Arg Thr Tyr Ala
305 310 315 320

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Gln Ser Ser Lys Cys Asn Lys Val His Lys Thr Trp Ser Trp Trp Thr
325 330 335

Leu Glu Ser Glu Ser Cys Arg Ser Ser Leu Pro Tyr Ile Cys Glu Met
340 345 350

Thr Ala Phe Arg Phe Pro Asp
355

<210> SEQ ID NO 33
<211> LENGTH: 661
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: LY64

<400> SEQUENCE: 33

Met Ala Phe Asp Val Ser Cys Phe Phe Trp Val Val Leu Phe Ser Ala
1 5 10 15

Gly Cys Lys Val Ile Thr Ser Trp Asp Gln Met Cys Ile Glu Lys Glu
20 25 30

Ala Asn Lys Thr Tyr Asn Cys Glu Asn Leu Gly Leu Ser Glu Ile Pro
35 40 45

Asp Thr Leu Pro Asn Thr Thr Glu Phe Leu Glu Phe Ser Phe Asn Phe
50 55 60

Leu Pro Thr Ile His Asn Arg Thr Phe Ser Arg Leu Met Asn Leu Thr
65 70 75 80

Phe Leu Asp Leu Thr Arg Cys Gln Ile Asn Trp Ile His Glu Asp Thr
85 90 95

Phe Gln Ser His His Gln Leu Ser Thr Leu Val Leu Thr Gly Asn Pro
100 105 110

Leu Ile Phe Met Ala Glu Thr Ser Leu Asn Gly Pro Lys Ser Leu Lys
115 120 125

His Leu Phe Leu Ile Gln Thr Gly Ile Ser Asn Leu Glu Phe Ile Pro
130 135 140

Val His Asn Leu Glu Asn Leu Glu Ser Leu Tyr Leu Gly Ser Asn His
145 150 155 160

Ile Ser Ser Ile Lys Phe Pro Lys Asp Phe Pro Ala Arg Asn Leu Lys
165 170 175

Val Leu Asp Phe Gln Asn Asn Ala Ile His Tyr Ile Ser Arg Glu Asp
180 185 190

Met Arg Ser Leu Glu Gln Ala Ile Asn Leu Ser Leu Asn Phe Asn Gly
195 200 205

Asn Asn Val Lys Gly Ile Glu Leu Gly Ala Phe Asp Ser Thr Val Phe
210 215 220

Gln Ser Leu Asn Phe Gly Gly Thr Pro Asn Leu Ser Val Ile Phe Asn
225 230 235 240

Gly Leu Gln Asn Ser Thr Thr Gln Ser Leu Trp Leu Gly Thr Phe Glu
245 250 255

Asp Ile Asp Asp Glu Asp Ile Ser Ser Ala Met Leu Lys Gly Leu Cys
260 265 270

Glu Met Ser Val Glu Ser Leu Asn Leu Gln Glu His Arg Phe Ser Asp
275 280 285

Ile Ser Ser Thr Thr Phe Gln Cys Phe Thr Gln Leu Gln Glu Leu Asp
290 295 300

Leu Thr Ala Thr His Leu Lys Gly Leu Pro Ser Gly Met Lys Gly Leu
305 310 315 320

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Asn Leu Leu Lys Lys Leu Val Leu Ser Val Asn His Phe Asp Gln Leu
 325 330 335
 Cys Gln Ile Ser Ala Ala Asn Phe Pro Ser Leu Thr His Leu Tyr Ile
 340 345 350
 Arg Gly Asn Val Lys Lys Leu His Leu Gly Val Gly Cys Leu Glu Lys
 355 360 365
 Leu Gly Asn Leu Gln Thr Leu Asp Leu Ser His Asn Asp Ile Glu Ala
 370 375 380
 Ser Asp Cys Cys Ser Leu Gln Leu Lys Asn Leu Ser His Leu Gln Thr
 385 390 395 400
 Leu Asn Leu Ser His Asn Glu Pro Leu Gly Leu Gln Ser Gln Ala Phe
 405 410 415
 Lys Glu Cys Pro Gln Leu Glu Leu Leu Asp Leu Ala Phe Thr Arg Leu
 420 425 430
 His Ile Asn Ala Pro Gln Ser Pro Phe Gln Asn Leu His Phe Leu Gln
 435 440 445
 Val Leu Asn Leu Thr Tyr Cys Phe Leu Asp Thr Ser Asn Gln His Leu
 450 455 460
 Leu Ala Gly Leu Pro Val Leu Arg His Leu Asn Leu Lys Gly Asn His
 465 470 475 480
 Phe Gln Asp Gly Thr Ile Thr Lys Thr Asn Leu Leu Gln Thr Val Gly
 485 490 495
 Ser Leu Glu Val Leu Ile Leu Ser Ser Cys Gly Leu Leu Ser Ile Asp
 500 505 510
 Gln Gln Ala Phe His Ser Leu Gly Lys Met Ser His Val Asp Leu Ser
 515 520 525
 His Asn Ser Leu Thr Cys Asp Ser Ile Asp Ser Leu Ser His Leu Lys
 530 535 540
 Gly Ile Tyr Leu Asn Leu Ala Ala Asn Ser Ile Asn Ile Ile Ser Pro
 545 550 555 560
 Arg Leu Leu Pro Ile Leu Ser Gln Gln Ser Thr Ile Asn Leu Ser His
 565 570 575
 Asn Pro Leu Asp Cys Thr Cys Ser Asn Ile His Phe Leu Thr Trp Tyr
 580 585 590
 Lys Glu Asn Leu His Lys Leu Glu Gly Ser Glu Glu Thr Thr Cys Ala
 595 600 605
 Asn Pro Pro Ser Leu Arg Gly Val Lys Leu Ser Asp Val Lys Leu Ser
 610 615 620
 Cys Gly Ile Thr Ala Ile Gly Ile Phe Phe Leu Ile Val Phe Leu Leu
 625 630 635 640
 Leu Leu Ala Ile Leu Leu Phe Phe Ala Val Lys Tyr Leu Leu Arg Trp
 645 650 655
 Lys Tyr Gln His Ile
 660

<210> SEQ ID NO 34
 <211> LENGTH: 429
 <212> TYPE: PRT
 <213> ORGANISM: Homo sapiens
 <220> FEATURE:
 <223> OTHER INFORMATION: FCRH1
 <400> SEQUENCE: 34

Met Leu Pro Arg Leu Leu Leu Leu Ile Cys Ala Pro Leu Cys Glu Pro
 1 5 10 15

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-continued

Ala Glu Leu Phe Leu Ile Ala Ser Pro Ser His Pro Thr Glu Gly Ser
 20 25 30
 Pro Val Thr Leu Thr Cys Lys Met Pro Phe Leu Gln Ser Ser Asp Ala
 35 40 45
 Gln Phe Gln Phe Cys Phe Phe Arg Asp Thr Arg Ala Leu Gly Pro Gly
 50 55 60
 Trp Ser Ser Ser Pro Lys Leu Gln Ile Ala Ala Met Trp Lys Glu Asp
 65 70 75 80
 Thr Gly Ser Tyr Trp Cys Glu Ala Gln Thr Met Ala Ser Lys Val Leu
 85 90 95
 Arg Ser Arg Arg Ser Gln Ile Asn Val His Arg Val Pro Val Ala Asp
 100 105 110
 Val Ser Leu Glu Thr Gln Pro Pro Gly Gly Gln Val Met Glu Gly Asp
 115 120 125
 Arg Leu Val Leu Ile Cys Ser Val Ala Met Gly Thr Gly Asp Ile Thr
 130 135 140
 Phe Leu Trp Tyr Lys Gly Ala Val Gly Leu Asn Leu Gln Ser Lys Thr
 145 150 155 160
 Gln Arg Ser Leu Thr Ala Glu Tyr Glu Ile Pro Ser Val Arg Glu Ser
 165 170 175
 Asp Ala Glu Gln Tyr Tyr Cys Val Ala Glu Asn Gly Tyr Gly Pro Ser
 180 185 190
 Pro Ser Gly Leu Val Ser Ile Thr Val Arg Ile Pro Val Ser Arg Pro
 195 200 205
 Ile Leu Met Leu Arg Ala Pro Arg Ala Gln Ala Ala Val Glu Asp Val
 210 215 220
 Leu Glu Leu His Cys Glu Ala Leu Arg Gly Ser Pro Pro Ile Leu Tyr
 225 230 235 240
 Trp Phe Tyr His Glu Asp Ile Thr Leu Gly Ser Arg Ser Ala Pro Ser
 245 250 255
 Gly Gly Gly Ala Ser Phe Asn Leu Ser Leu Thr Glu Glu His Ser Gly
 260 265 270
 Asn Tyr Ser Cys Glu Ala Asn Asn Gly Leu Gly Ala Gln Arg Ser Glu
 275 280 285
 Ala Val Thr Leu Asn Phe Thr Val Pro Thr Gly Ala Arg Ser Asn His
 290 295 300
 Leu Thr Ser Gly Val Ile Glu Gly Leu Leu Ser Thr Leu Gly Pro Ala
 305 310 315 320
 Thr Val Ala Leu Leu Phe Cys Tyr Gly Leu Lys Arg Lys Ile Gly Arg
 325 330 335
 Arg Ser Ala Arg Asp Pro Leu Arg Ser Leu Pro Ser Pro Leu Pro Gln
 340 345 350
 Glu Phe Thr Tyr Leu Asn Ser Pro Thr Pro Gly Gln Leu Gln Pro Ile
 355 360 365
 Tyr Glu Asn Val Asn Val Val Ser Gly Asp Glu Val Tyr Ser Leu Ala
 370 375 380
 Tyr Tyr Asn Gln Pro Glu Gln Glu Ser Val Ala Ala Glu Thr Leu Gly
 385 390 395 400
 Thr His Met Glu Asp Lys Val Ser Leu Asp Ile Tyr Ser Arg Leu Arg
 405 410 415
 Lys Ala Asn Ile Thr Asp Val Asp Tyr Glu Asp Ala Met
 420 425

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<210> SEQ ID NO 35
<211> LENGTH: 977
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: IRTA2

<400> SEQUENCE: 35

Met Leu Leu Trp Val Ile Leu Leu Val Leu Ala Pro Val Ser Gly Gln
 1             5             10             15

Phe Ala Arg Thr Pro Arg Pro Ile Ile Phe Leu Gln Pro Pro Trp Thr
      20             25             30

Thr Val Phe Gln Gly Glu Arg Val Thr Leu Thr Cys Lys Gly Phe Arg
      35             40             45

Phe Tyr Ser Pro Gln Lys Thr Lys Trp Tyr His Arg Tyr Leu Gly Lys
 50             55             60

Glu Ile Leu Arg Glu Thr Pro Asp Asn Ile Leu Glu Val Gln Glu Ser
 65             70             75             80

Gly Glu Tyr Arg Cys Gln Ala Gln Gly Ser Pro Leu Ser Ser Pro Val
      85             90             95

His Leu Asp Phe Ser Ser Ala Ser Leu Ile Leu Gln Ala Pro Leu Ser
      100            105            110

Val Phe Glu Gly Asp Ser Val Val Leu Arg Cys Arg Ala Lys Ala Glu
      115            120            125

Val Thr Leu Asn Asn Thr Ile Tyr Lys Asn Asp Asn Val Leu Ala Phe
      130            135            140

Leu Asn Lys Arg Thr Asp Phe His Ile Pro His Ala Cys Leu Lys Asp
 145            150            155            160

Asn Gly Ala Tyr Arg Cys Thr Gly Tyr Lys Glu Ser Cys Cys Pro Val
      165            170            175

Ser Ser Asn Thr Val Lys Ile Gln Val Gln Glu Pro Phe Thr Arg Pro
      180            185            190

Val Leu Arg Ala Ser Ser Phe Gln Pro Ile Ser Gly Asn Pro Val Thr
      195            200            205

Leu Thr Cys Glu Thr Gln Leu Ser Leu Glu Arg Ser Asp Val Pro Leu
      210            215            220

Arg Phe Arg Phe Phe Arg Asp Asp Gln Thr Leu Gly Leu Gly Trp Ser
 225            230            235            240

Leu Ser Pro Asn Phe Gln Ile Thr Ala Met Trp Ser Lys Asp Ser Gly
      245            250            255

Phe Tyr Trp Cys Lys Ala Ala Thr Met Pro His Ser Val Ile Ser Asp
      260            265            270

Ser Pro Arg Ser Trp Ile Gln Val Gln Ile Pro Ala Ser His Pro Val
      275            280            285

Leu Thr Leu Ser Pro Glu Lys Ala Leu Asn Phe Glu Gly Thr Lys Val
      290            295            300

Thr Leu His Cys Glu Thr Gln Glu Asp Ser Leu Arg Thr Leu Tyr Arg
 305            310            315            320

Phe Tyr His Glu Gly Val Pro Leu Arg His Lys Ser Val Arg Cys Glu
      325            330            335

Arg Gly Ala Ser Ile Ser Phe Ser Leu Thr Thr Glu Asn Ser Gly Asn
      340            345            350

Tyr Tyr Cys Thr Ala Asp Asn Gly Leu Gly Ala Lys Pro Ser Lys Ala
      355            360            365

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Val	Ser	Leu	Ser	Val	Thr	Val	Pro	Val	Ser	His	Pro	Val	Leu	Asn	Leu
370						375					380				
Ser	Ser	Pro	Glu	Asp	Leu	Ile	Phe	Glu	Gly	Ala	Lys	Val	Thr	Leu	His
385					390					395					400
Cys	Glu	Ala	Gln	Arg	Gly	Ser	Leu	Pro	Ile	Leu	Tyr	Gln	Phe	His	His
				405					410					415	
Glu	Asp	Ala	Ala	Leu	Glu	Arg	Arg	Ser	Ala	Asn	Ser	Ala	Gly	Gly	Val
				420				425					430		
Ala	Ile	Ser	Phe	Ser	Leu	Thr	Ala	Glu	His	Ser	Gly	Asn	Tyr	Tyr	Cys
		435					440					445			
Thr	Ala	Asp	Asn	Gly	Phe	Gly	Pro	Gln	Arg	Ser	Lys	Ala	Val	Ser	Leu
		450				455					460				
Ser	Ile	Thr	Val	Pro	Val	Ser	His	Pro	Val	Leu	Thr	Leu	Ser	Ser	Ala
465					470					475					480
Glu	Ala	Leu	Thr	Phe	Glu	Gly	Ala	Thr	Val	Thr	Leu	His	Cys	Glu	Val
				485					490					495	
Gln	Arg	Gly	Ser	Pro	Gln	Ile	Leu	Tyr	Gln	Phe	Tyr	His	Glu	Asp	Met
			500					505					510		
Pro	Leu	Trp	Ser	Ser	Ser	Thr	Pro	Ser	Val	Gly	Arg	Val	Ser	Phe	Ser
		515					520					525			
Phe	Ser	Leu	Thr	Glu	Gly	His	Ser	Gly	Asn	Tyr	Tyr	Cys	Thr	Ala	Asp
		530				535					540				
Asn	Gly	Phe	Gly	Pro	Gln	Arg	Ser	Glu	Val	Val	Ser	Leu	Phe	Val	Thr
545					550					555					560
Val	Pro	Val	Ser	Arg	Pro	Ile	Leu	Thr	Leu	Arg	Val	Pro	Arg	Ala	Gln
				565					570					575	
Ala	Val	Val	Gly	Asp	Leu	Leu	Glu	Leu	His	Cys	Glu	Ala	Pro	Arg	Gly
			580					585					590		
Ser	Pro	Pro	Ile	Leu	Tyr	Trp	Phe	Tyr	His	Glu	Asp	Val	Thr	Leu	Gly
		595					600					605			
Ser	Ser	Ser	Ala	Pro	Ser	Gly	Gly	Glu	Ala	Ser	Phe	Asn	Leu	Ser	Leu
		610				615					620				
Thr	Ala	Glu	His	Ser	Gly	Asn	Tyr	Ser	Cys	Glu	Ala	Asn	Asn	Gly	Leu
625					630					635					640
Val	Ala	Gln	His	Ser	Asp	Thr	Ile	Ser	Leu	Ser	Val	Ile	Val	Pro	Val
				645					650					655	
Ser	Arg	Pro	Ile	Leu	Thr	Phe	Arg	Ala	Pro	Arg	Ala	Gln	Ala	Val	Val
			660					665					670		
Gly	Asp	Leu	Leu	Glu	Leu	His	Cys	Glu	Ala	Leu	Arg	Gly	Ser	Ser	Pro
		675					680					685			
Ile	Leu	Tyr	Trp	Phe	Tyr	His	Glu	Asp	Val	Thr	Leu	Gly	Lys	Ile	Ser
		690				695					700				
Ala	Pro	Ser	Gly	Gly	Gly	Ala	Ser	Phe	Asn	Leu	Ser	Leu	Thr	Thr	Glu
705					710					715					720
His	Ser	Gly	Ile	Tyr	Ser	Cys	Glu	Ala	Asp	Asn	Gly	Pro	Glu	Ala	Gln
				725					730					735	
Arg	Ser	Glu	Met	Val	Thr	Leu	Lys	Val	Ala	Val	Pro	Val	Ser	Arg	Pro
			740					745					750		
Val	Leu	Thr	Leu	Arg	Ala	Pro	Gly	Thr	His	Ala	Ala	Val	Gly	Asp	Leu
		755					760					765			
Leu	Glu	Leu	His	Cys	Glu	Ala	Leu	Arg	Gly	Ser	Pro	Leu	Ile	Leu	Tyr
		770				775					780				

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Arg Phe Phe His Glu Asp Val Thr Leu Gly Asn Arg Ser Ser Pro Ser
785 790 795 800

Gly Gly Ala Ser Leu Asn Leu Ser Leu Thr Ala Glu His Ser Gly Asn
805 810 815

Tyr Ser Cys Glu Ala Asp Asn Gly Leu Gly Ala Gln Arg Ser Glu Thr
820 825 830

Val Thr Leu Tyr Ile Thr Gly Leu Thr Ala Asn Arg Ser Gly Pro Phe
835 840 845

Ala Thr Gly Val Ala Gly Gly Leu Leu Ser Ile Ala Gly Leu Ala Ala
850 855 860

Gly Ala Leu Leu Leu Tyr Cys Trp Leu Ser Arg Lys Ala Gly Arg Lys
865 870 875 880

Pro Ala Ser Asp Pro Ala Arg Ser Pro Pro Asp Ser Asp Ser Gln Glu
885 890 895

Pro Thr Tyr His Asn Val Pro Ala Trp Glu Glu Leu Gln Pro Val Tyr
900 905 910

Thr Asn Ala Asn Pro Arg Gly Glu Asn Val Val Tyr Ser Glu Val Arg
915 920 925

Ile Ile Gln Glu Lys Lys Lys His Ala Val Ala Ser Asp Pro Arg His
930 935 940

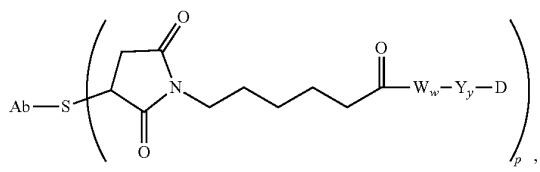
Leu Arg Asn Lys Gly Ser Pro Ile Ile Tyr Ser Glu Val Lys Val Ala
945 950 955 960

Ser Thr Pro Val Ser Gly Ser Leu Phe Leu Ala Ser Ser Ala Pro His
965 970 975

Arg

What is claimed is:

1. An antibody-drug conjugate having the formula:



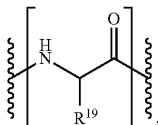
or a pharmaceutically acceptable salt thereof, wherein:

Ab is an antibody,

S is sulfur,

each $-W_y-$ unit is a tetrapeptide; wherein each

$-W-$ unit is independently an Amino Acid unit having the formula denoted below in the square bracket:



wherein R^{19} is hydrogen or benzyl,

Y is a Spacer unit,

y is 0, 1 or 2,

D is a drug moiety, and

p ranges from 1 to about 20,

wherein the S is a sulfur atom on a cysteine residue of the antibody, and

wherein the drug moiety is intracellularly cleaved in a patient from the antibody of the antibody-drug conjugate or an intracellular metabolite of the antibody-drug conjugate.

2. The antibody-drug conjugate of claim 1, wherein Y is a self-immolative spacer.

3. The antibody-drug conjugate of claim 2, wherein y is 1.

4. The antibody-drug conjugate of claim 3, wherein p is about 3 to about 8.

5. The antibody-drug conjugate of claim 4, wherein p is about 8.

6. The antibody-drug conjugate of claim 1, 2, 3, 4, or 5, wherein the bioavailability of the antibody-drug conjugate or an intracellular metabolite of the antibody-drug conjugate in a patient is improved when compared to a drug compound comprising the drug moiety of the antibody-drug conjugate.

7. The antibody-drug conjugate compound of claim 1, 2, 3, 4, or 5, wherein the bioavailability of the antibody-drug conjugate or an intracellular metabolite of the antibody-drug conjugate in a patient is improved when compared to an analog of the antibody-drug conjugate not having the drug moiety.

8. The antibody-drug conjugate compound of claim 1, 2, 3, 4, or 5, wherein the drug moiety is intracellularly cleaved in a patient from an intracellular metabolite of the antibody-drug conjugate.

9. The antibody-drug conjugate of claim 1, 2, 3, 4, or 5, wherein the antibody is a monoclonal antibody.

10. The antibody-drug conjugate of claim 9, wherein the antibody is a humanized monoclonal antibody.

* * * * *

FORM 19. Certificate of Compliance with Type-Volume Limitations

Form 19
July 2020

**UNITED STATES COURT OF APPEALS
FOR THE FEDERAL CIRCUIT**

CERTIFICATE OF COMPLIANCE WITH TYPE-VOLUME LIMITATIONS

Case Number: 2023-2424, 2024-1176

Short Case Caption: Seagen Inc. v. Daiichi Sankyo Company, Ltd.

Instructions: When computing a word, line, or page count, you may exclude any items listed as exempted under Fed. R. App. P. 5(c), Fed. R. App. P. 21(d), Fed. R. App. P. 27(d)(2), Fed. R. App. P. 32(f), or Fed. Cir. R. 32(b)(2).

The foregoing filing complies with the relevant type-volume limitation of the Federal Rules of Appellate Procedure and Federal Circuit Rules because it meets one of the following:

- ☒ the filing has been prepared using a proportionally-spaced typeface and includes 13,821 words.
- ☐ the filing has been prepared using a monospaced typeface and includes _____ lines of text.
- ☐ the filing contains _____ pages / _____ words / _____ lines of text, which does not exceed the maximum authorized by this court's order (ECF No. _____).

Date: 03/22/2024

Signature: /s/ Christopher N. Sipes

Name: Christopher N. Sipes

FORM 31. Certificate of Confidential Material

Form 31
July 2020

**UNITED STATES COURT OF APPEALS
FOR THE FEDERAL CIRCUIT**

CERTIFICATE OF CONFIDENTIAL MATERIAL

Case Number: 2023-2424, 2024-1176

Short Case Caption: Seagen Inc. v. Daiichi Sankyo Company, Ltd.

Instructions: When computing a confidential word count, Fed. Cir. R. 25.1(d)(1)(C) applies the following exclusions:

- Only count each unique word or number once (repeated uses of the same word do not count more than once).
- For a responsive filing, do not count words marked confidential for the first time in the preceding filing.

The limitations of Fed. Cir. R. 25.1(d)(1) do not apply to appendices; attachments; exhibits; and addenda. *See* Fed. Cir. R. 25.1(d)(1)(D).

The foregoing document contains 9 number of unique words (including numbers) marked confidential.

- ☒ This number does not exceed the maximum of 15 words permitted by Fed. Cir. R. 25.1(d)(1)(A).
- ☐ This number does not exceed the maximum of 50 words permitted by Fed. Cir. R. 25.1(d)(1)(B) for cases under 19 U.S.C. § 1516a or 28 U.S.C. § 1491(b).
- ☐ This number exceeds the maximum permitted by Federal Circuit Rule 25.1(d)(1), and the filing is accompanied by a motion to waive the confidentiality requirements.

Date: 03/22/2024

Signature: /s/ Christopher N. Sipes

Name: Christopher N. Sipes